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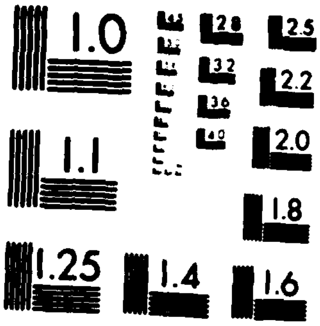

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LOW WATER ACTIVITY PACKAGED WHITE BREAD

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U.S. Army Natick R & D Center
Kansas Street
Natick, MA 01760

Principal Investigator: Dr. Henry K. Leung
Co-investigators: Dr. Charles W. Nagel
Mr. Gordon G. Rubenthaler*
Research Associates: Dr. Debbie Hsu
Ms. Nancy Larson-Powers
Dr. A. Abdelrahman
Technical Assistants: Ms. Dorothy MacEachern
Mr. Mike Costello

Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164-6330

*Western Wheat Quality Laboratory, USDA, Pullman, WA 99164-4004

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SUMMARY

The purpose of this study was to develop a shelf-stable packaged white pan bread for military use. Shelf life of bread may be extended to several months by proper control of water activity, pH, packaging conditions, and by addition of antimicrobial compounds and dough conditioners. Water activity of bread was effectively lowered from 0.97 to 0.92-0.93 by addition of 7% sorbitol, 1% propylene glycol and 2% glycerol (flour basis). Incorporation of 0.3% lactic acid powder to the bread reduced its pH to 5.0. Other formulation changes included 10% shortening and addition of sodium stearoyl-2-lactylate (anti-staling). The resulting humectant bread was satisfactory for baking and sensory quality. To prevent dehydration and to exclude oxygen, the bread was packaged in a partial CO_2 atmosphere in a retort pouch.

The packaged humectant bread was microbiologically stable up to about four months. Growth of Aspergillus flavus, Staphylococcus aureus and other aerobic organisms were inhibited. However, pH of the product should be lowered to 4.8 to guard against anaerobic organisms.

Storage of the humectant bread resulted in a number of undesirable changes: increases in browning, firmness, dryness, crumbliness, rancid flavor and aroma, and decreases in pH and overall preference. Further improvement will be needed to extend the shelf-life of the bread to one year at 30°C.

Based on this study, a maximum a_w of 0.92-0.93 and a maximum pH of 4.8 are recommended for the humectant bread. Partial CO_2 packaging using a retort pouch or metal can is preferred for maximum stability.

INTRODUCTION

Regular United States white pan bread is susceptible to microbial spoilage because of its relatively high moisture content (35%). Even with added preservatives such as calcium propionate, packaged white bread usually spoils within two weeks at room temperature. In the early 1940's, a shelf-stable canned white bread was developed for military uses. This was accomplished by baking the bread in a can and sealing the container while it was still hot. When the bread cooled, a vacuum was created inside the can. The combination of heat treatment, exclusion of oxygen, as well as a reduction in pH (< 4.8) and moisture content (< 35%) contributed to the microbial stability of the product (Matz, 1972). However, the bread produced by this method is different in quality from the normal white pan bread.

It has been well recognized that water activity is a key factor in controlling microbial spoilage of food. Water activity of a material is defined as:

$$a_w = Pi/Po$$

Where Pi = vapor pressure of water in the atmosphere in equilibrium with the material

Po = vapor pressure of pure water at the same temperature

In general, most bacteria, yeasts and molds do not grow below a_w of 0.90, 0.87 and 0.80, respectively (Troller, 1979). Bread crumb has a water activity of about 0.97, and is therefore susceptible to microbial spoilage.

Water activity of a food material can be effectively lowered by the addition of water binders or humectants. The common humectants include salt, sugars, gums and polyhydric alcohols (polyols) such as glycerol, sorbitol and propylene glycol. The polyols are more effective water binders than sugars. Propylene glycol has a bitter-sweet taste; glycerol is slightly sweet and sorbitol is sweet. In addition, sorbitol has a laxative effect if consumed at high concentration. In baked goods, sorbitol can be used at a concentration up to 30%, and propylene glycol is limited to 2%. Glycerol is in the Generally Recognized As Safe (GRAS) list.

Besides water activity, other factors such as pH, oxygen, antimicrobial agents, heat treatment and packaging are also important in controlling microbial growth. The purpose of this project was to develop a shelf-stable white pan bread in a flexible pouch by utilizing the above factors for preservation. The specific objectives of this project were:

(1) To investigate the effect of humectants on bread making properties.
(2) To determine the effects of different ingredients on water activity, pH, sensory properties, staling and microbial stability of bread.
(3) To develop an acceptable, shelf-stable white pan bread by controlling its water activity, pH and packaging environment.
(4) To conduct a storage stability study of the final products.

MATERIALS AND METHODS

Baking

Laboratory

The wheat flour used in this study was a blend of Montana hard wheat flour with 12.6% moisture, 12.8% protein and 0.44% ash. The straight dough method was used throughout the study except where indicated otherwise. The basic formula included the following ingredients unless otherwise specified: 100 g flour (14% moisture basis), 5 g yeast, 6 g sucrose, 3 g shortening (Crisco), 1.5 g salt and 0.25 g malt. Sorbitol (Sigma Chemical Co.), glycerol (Sigma Chemical Co.), propylene glycol (Baker CHemical Co.), and sodium stearoyl-2-lactylate (SSL) (Patco Products, Kansas City, MO), were added at different concentrations. Baking absorption (g water/100g flour), mixing time and oxidation were optimized for each formula. The fermentation and proofing (35°C) times were adjusted for different formulas. Breads were baked at 204.4°C for 26-28 min, and weighed immediately after removal from the oven. Loaf volume was determined by rapeseed displacement.

Local Bakery

The humectant breads were produced in a local bakery using the straight dough method. The formulas for the bread are summarized in Table 1. The flour was a blend of all purpose flours containing 12.7% protein (14% m.b.). Lactic acid powder (Purac), sorbitol (Sigma Chemical Co.), glycerol (Sigma Chemical Co.), propylene glycol (Baker Chemical Co.) and sodium stearoyl-2-lactylate (SSL) (Patco Products, Kansas City, MO) were added to different concentrations. Baking absorption (g water/100 g flour), mixing time and oxidation were optimized for each formulation.

The ingredients, except water, were blended for five minutes. Water was added gradually to the mixture while the dough was mixed to optimum consistency. The dough was fermented at room temperature, divided (scaled) and rounded by hand. After molding, the dough was panned and placed inside the proofing cabinet. Proofing was completed when the top of the dough reached 1/2 inch above the rim of the pan. The breads were baked at 375° and cooled for 3 hr at room temperature before packing and delivery to Washington State University. The baking procedures of the humectant breads are summarized in Table 2.

Physical and Chemical Analysis

Moisture and Water Activity

Moisture content of bread crumb and crust was determined using a modification of AACC method 44-4D. One (1) g of sample was placed in a glass petri dish, weighed and placed in a vacuum oven at 70°C for 24 hours.

Water activity of bread crumb or crust was determined using a SC-10 Thermocouple Psychrometer (Duagon Devices, Pullman, WA). The instrument consisted of a sample changer, a chromel constantan thermocouple, and a microvoltmeter (100 m V sensitivity). The sample changer can hold up to nine sample and one water cups. Samples were taken from the center portion of the loaf from crumb and the bottom crust near the end for crust. Each sample was firmly packed into the sample cup until it was about half full. A sample of saturated salt solution of potassium nitrate (KNO_3) was included for determining the cell constant. After the samples were loaded, they were allowed to equilibrate for at least 1 hour. When a reading was taken, the thermocouple was wetted, then placed above the desired sample and the chamber sealed. The voltage output was read at 2 minutes and chamber temperature noted. Water activity was computed using an Apple II computer according to the Instruction Manual.

Table 1. Formulas of the humectant breads baked in a commercial bakery

	% Flour Weight	
Ingredient	7-1-2[a]	6-1.5-2[a]
Flour (14% mb; 12.7% protein)	100	100
Water	47	57
Yeast (Compressed)	5.25	5.25
Shortening	10	10
Sugar	3	3
Salt	1.5	1.5
Ascorbic Acid	(40 ppm)	(40 ppm)
Potassium bromate	(10 ppm)	(10 ppm)
Malt	-	-
Sodium stearoyl lactylate	0.5	0.5
Calcium propionate	0.3	0.3
Lactic acid (60% powder)	0.3	0.3
Sorbitol	7	6
Propylene glycol	1	1.5
Glycerol	2	2

[a] % sorbitol - % propylene glycol - % glycerol

Table 2. Baking procedures and times (min) of the humectant breads produced in a commercial bakery

Steps	7-1-2[a]	6-1.5-2[a]
Mixing	30	30
First Fermentation	38	14
Dividing and Rounding	21	27
Second Fermentation	5	5
Molding and Panning	12	12
Proofing (93°F, 70% RH)	83	102
Baking (375°F)	30	30
Cooling	180	180

[a] % sorbitol - % propylene glycol - % glycerol

pH

The pH of bread crumb was determined according to AACC Method 02-52 (AACC, 1984). The bread crumb was disintegrated into particles using a blender. A 10 g sample was added to 100 g distilled water in a beaker and mixed thoroughly before pH measurement.

Color

A Hunter Color and Difference Meter (Model D-25 D, Hunterlab, Fairfax, VA) was standardized to the white tile prior to determining the L, a, b values of bottom crust and crumb.

Firmness

Firmness of bread crumb was determined with a Fudoh Rheometer model NRM-2002J (Fudoh Kohyo, Co., Tokyo, Japan). A custom-built adapter consisting of a stainless steel rod with a circular lucite plate (32 mm diameter) attached to the end was used for the test. A 2 cm cube sample was removed from the center of the 6 slices taken from the center of the loaf (the seventh slice which was cut first was discarded because of width variation). Each sample was compressed to a height of 0.6 cm using a crosshead speed of 2 cm/min and grams force was recorded.

Thermal Analysis

A Perkin-Elmer (Norwalk, CT) Differential Scanning Calorimeter (DSC-4) equipped with the Data Analysis Station was used to determine the starch crystallinity of the bread. A No. 1 Cork Borer was used to remove a plug of bread crumb from the center of the loaf. The plug was compressed and a 5-6 mg slice was removed using a scalpel. The sample was placed in a volatile sample pan and weighed. Two parts of water (by weight) was added to the sample. The pan was sealed with a volatile sample sealer and allowed to equilibrate at room temperature for at least one hour before the thermal analysis.

The temperature scale of the DSC was calibrated using pure indium (M.P. = 156.6°C). An automatic device was built into the instrument for heat flow calibration. A pan containing 10 mg water was used as the reference. The sample was scanned from 20-100°C at a rate of 10°C/min. The onset temperature and peak area of the curve were determined using the Perkin-Elmer TADS DSC-4 Standard Software Program.

Headspace Analysis

Carbon dioxide content of the headspace in the packaged samples was determined using a Beckman GC-2A Gas Chromatograph (Beckman Instruments, Inc., Irvine, CA) equipped with a thermal conductivity detector, a Beckman Col System Ser CS41 Column and a Spectraphysics integrator. Mixtures of CO_2 and air were used to prepare the standard curves. A 5 ml gas sample was withdrawn from the bag through a silicon system and injected into the GC. The ratio of CO_2 to O_2 was used to determine the percent CO_2 present in the pouch.

Humectant Analysis

Sorbitol, glycerol and propylene glycol of the humectant breads were determined using the high performance liquid chromatography (HPLC) method developed in our laboratory (Nagel et al., 1981). Duplicate bread slice samples of about 30 g each were removed from the center of two separate loaves of bread. The sample was homogenized with water in a Waring Blender for one minute, and made up to 250 ml. The mixture was centrifuged at 10,000 x g for 5 min. Twenty five milliliters of supernate was run through an Amberlite TR 120 H^+ column and then an Amberlite IR 45 OH^- column. The sample was made up to 100 ml with distilled water, filtered and analyzed by HPLC (Nagel et al., 1981).

Sensory Evaluation

An untrained panel, consisting of 38 graduate students and staff members at Washington State University, was used to conduct the preference test of the bread samples. A nine point hedonic scale (Larmond, 1970) was used for overall acceptance. A copy of the score card is given in Appendix A. The bread was sliced and kept in plastic bags before serving. The samples were coded and randomized in order of presentation. Each judge was presented five samples: control, 5% sorbitol, 20% sorbitol, 8% glycerol, and 10% sorbitol-5% glycerol-2% propylene glycol. The data were analyzed statistically for any significant differences.

To evaluate the effect of different humectants on bread quality, a semi-trained panel consisting of ten judges was used. Most of the judges were members of the Western Wheat Quality Laboratory. After cooling, six slices were taken from each loaf of bread, cut into halves, and placed in plastic wrap to prevent dehydration. The panelists were asked to evaluate crumb texture, off-flavor and overall preference (Prentice and D'Appolonia, 1977). A copy of the scorecard is given in Appendix B.

To select a descriptive analysis panel, nineteen members of the Department of Food Science and Human Nutrition at Washington State University were given a series of nine triangle tests. Panelists were screened for their ability to differentiate between various commercial white bread samples. Twelve finalists and one alternate (10 males and 3 females) were selected to participate in further training.

Panelists were trained for the first three one-hour training sessions, the judges evaluated the samples, listing as many texture, aroma and flavor descriptors as possible. Then the group discussed and developed definitions for these terms. The evaluation was broken down into appearance, aroma, texture determined by fingers, mouthfeel textural characteristics, crumb flavor and crust flavor. The judges were also allowed to express an overall preference; however, these results were different from consumers' since the judges were highly trained and sensitive to small differences not detected by consumers.

In the fourth through seventh sessions, the judges evaluated samples using the scorecard developed from the terms derived in previous sessions, and discussed how the evaluation was done. In the final three training sessions, the judges evaluated four samples on a scorecard. The sample consisted of a slice of 100 g loaf bread (12 mm thick) placed in a Ziploc bag and labeled with a 3-digit code. The testing was replicated three times with (1.) one-day old 5-2-2 (5% sorbitol - 2% propylene glycol - 2% glycerol) bread, (2.) six-month old 5-2-2 bread, (3.) one-day old 1-4-4 bread, and (4.) one-day old 8-2-2 bread. The data from these evaluations were analyzed statistically to determine how the judges performed individually and as a group. Finally, the judges participated in a one-hour retraining session where the scorecard (No. 2) was modified (Appendix C).

Scorecard No. 2 was used for the descriptive analysis of breads by the trained panel. A preference test was conducted when the bread had been stored five weeks. An untrained panel consisting of 32 people from Washington State University evaluated preference using an unstructured hedonic scale. A copy of the scorecard for the preference test is given in Appendix D.

The bread samples were sliced with a Bosch Kitchen Slicer, cut into halves and placed in Ziploc bags (Dow chemical) before serving. The samples were coded with 3-digit numbers and presented in a balanced complete block design. The judges were presented with the samples one at a time, with a spit cup, water and a scorecard. The data were analyzed for statistical significance by analysis of variance.

Microbial Studies

Standard Plate Count (SPC) and anaerobic count were conducted according to the standard procedures (Speck, 1976). Eleven grams of sample were placed in a sterile polyethylene bag (18 cm x 30 cm) with 99 ml of dilution water. The sample was homogenized in a Stomacher Lab Blender Model 400 (Seward Laboratory, London) before microbial analysis. The anaerobic count was determined according to the most probable number (MPN) method.

For the challenge studies, Penicillium expansum, Aspergillus niger, and Clostridium sporogenes cultures were obtained from the culture collection of the Department of Food Science & Human Nutrition at Washington State University. The Staphylococcus aureus culture was obtained from the American Type Culture Collection (ATCC 13565).

The molds used for the challenge study were grown on regular bread slices at 30°C. When the bread slices were overgrown with mold spores, they were freeze dried and ground into powder using a mortar and pestle. About 2 mg of the mold powder was transferred to a one-ml ampule, flushed with carbon dioxide and sealed. The breads were baked as described previously and placed into metallized polyester (2.5 mil) bags (Kapak Corporation, Bloomington, MN). An ampule containing the mold spores was placed inside each bag containing the bread sample. The bags were evacuated and flushed with carbon dioxide twice before heat sealing. After sealing, the ampule inside each bag was broken and the bag was shaken to allow distribution of mold spores on the bread surface. The bags were stored at 30°C and removed periodically for observation of visible mold growth.

The C. sporogenes spores used for the challenge study were prepared according to Vareltzis et al. (1984). The spore suspension was mixed with sterilized wheat flour and freeze-dried. The spores and vegetative cells were enumerated using the PE-2 medium (Speck, 1976). To inoculate the bread, the spore-flour mixture was added to the dough before mixing. The inoculum size was approximately 1000 spores/g flour. The breads were baked, cooled, sealed in metallized polyester bags with a carbon dioxide flush, and stored at 30°C. Samples were removed periodically for spore and repetitive cell counts.

The Staphylococcus aureus culture was grown overnight on T-soy slant and washed off with 7 ml sterile water. A series of cell suspensions was prepared by dilution and the turbidity of the samples was determined using a Klett-Summerson Photoelectric Colorimeter (Klett Mfg. Co., N.Y.). Concentration of the culture was determined from a standard curve relating cell count to Klett (turbidity) units. An inoculum containing about 10^5 CFU/ml was prepared by dilution and 0.1 ml of the culture was applied onto the bread crust in a circular area of about 4 cm in diameter at one end of the loaf. After incubation, the crust portion containing the inoculum was removed and mixed with 100 ml sterile saline using a Stomacher. It was enumerated using Staph-110 Agar (Difco, Detroit, MI).

Storage Studies

After the bread was delivered, they were repackaged in retort pouches made of polyester-foil-polyethylene laminate (Kapak Corp., Bloomington, MN) to which a silicon septum had been added. Except for the samples designated for storage in air, the bags were evacuated and flushed with CO_2 twice before heat sealing using a Kenfield Vacuum Sealer (International Kenfield Distributing Co., Chicago, IL). The breads were stored at -20, 20, 30 and 45°C, and duplicate samples were removed for analysis periodically.

When the breads were removed from storage for analysis, one quarter of the loaf was cut off by electric knife (Hamilton Beach, Scovill Division, Waterbury, CT) then the bottom 1/2" of the loaf was removed and stored in a Ziploc bag (Dow

Chemical, Indianapolis, IN) for color evaluation. Seven 2-cm thick slices were removed with a Bosch food slicer (Model EAS 55, Robert Bosch, Hausgerate GMBI, Munich, West Germany) for firmness measurement. Moisture, water activity and pH were determined on the quarter endpieces of the loaf.

RESULTS AND DISCUSSION

(I) Effects of Humectants on Baking, Sensory and Physical Properties of Bread.

Dough Mixing Properties

In breadmaking, the first step is mixing of the ingredients into a dough. The ultimate quality of the bread is closely related to the mixing characteristics of the flour dough. Humectants may change the dough properties by imbibing water and interacting with other components.

Addition of sorbitol up to 20% had little effect on water absorption, but increased the mixing time of the dough (Figure 1). Unlike sorbitol, glycerol (20%) did not affect the mixing time, but caused a decrease in water absorption form 65 to 57%. Propylene glycol was detrimental to dough properties even at low concentrations (2-5%). The flour dough containing 5% propylene glycol showed a decrease in dough consistency and an increase in stickiness.

Baking Properties

Loaf volume and water activity (a_w) of bread crumb decreased with increasing sorbitol and glycerol concentrations in bread (Figure 2). The effects were more pronounced with glycerol than with sorbitol. For instance, addition of 15% sorbitol to bread lowered the a_w from about 0.97 to 0.94 and the loaf volume from 990 c.c. to 960 c.c. By comparison, 8% glycerol was far more detrimental to loaf volume than 15% sorbitol although both humectants at the specified concentration would achieve the same effect in depressing a_w of the bread crumb.

Additional data showing the humectant effect on baking properties and sensory quality of bread are presented in Table 3. Increased humectant concentrations resulted in longer mixing time, longer proof time and decreased loaf volume. Water activity of bread crumb ranged from 0.907 for the 4-4-4 (4% sorbitol-4% glycerol-4% propylene glycol) to 0.939 for the 4-1-1 sample (Table 4).

Sensory Properties

The first sensory preference test was conducted using a panel of 38 judges. The samples evaluated were: control, 5% sorbitol, 20% sorbitol, 8% glycerol, and 10% sorbitol-5% glycerol-2% propylene glycol. A nine point hedonic scale (1 = dislike extremely, 9 = like extremely) was used. There was no significant difference in overall acceptability between the control and the breads containing humectants. The preference scores were in the range of 6.2 - 6.7, corresponding to "like slightly" or "like moderately". The results are somewhat unexpected since the humectants impart a certain bitter-sweet flavor to foods (Karel, 1973). Some panelists may have preferred the humectant breads because of the sweetness.

The ten samples shown in Table 3 were evaluated by a separate panel and the results are summarized in Table 4. The best flavor scores (4.0 - 4.5) were obtained with the 1-1-4, 4-1-1, 8-1-2, 1-2-1 and 4-2-1 samples. Taking into consideration a_w, loaf volume and sensory scores, a humectant combination similar to 8-1-2 seemed to be the best choice. Since the original canned bread contained only 7% sorbitol, the 7-1-2 combination was selected for the storage studies.

Table 3. Baking data of bread made with different concentrations of humectants.

Sorb. (%)	P.G. (%)	Gly. (%)	Absorption (%)	Mix Time (min:sec)	Ferment Time (Min)	Proof Time (Min)	Loaf[a] Wt. (g)	Loaf[a] Vol. (cc)	Crumb[b] Grain
Control			65	2:40	90	30	141.2	980	6
1	1	4	59	3:45	103	42	156.3	950	5
4	1	1	61	4:10	103	35	159.8	908	6
8	1	2	59	5:00	103	42	160.6	940	6
1	2	1	59	3:50	103	38	155.1	923	5
4	2	1	59	3:55	103	42	158.3	945	6
4	2	2	59	4:20	103	40	157.8	903	5
4	2	4	59	4:10	103	50	159.5	890	5
8	2	4	59	5:10	103	56	163.0	910	5
1	4	2	59	4:00	103	46	158.3	913	4
4	4	4	59	4:30	103	64	161.6	890	2
8	4	1	59	4:30	103	63	164.9	925	3

[a] Mean of duplicate samples

[b] 9 = excellent, 1 = poor

Table 4. Effect of humectants on water activity and sensory properties of

Sorb. (%)	P.G. (%)	Gly. (%)	Crumb[a] Grain	Flavor[b]	Crumb[c] Texture	Overall[d] Preference	a_w[e]
1	1	4	5	4.0	2.5	3.5	0.934
4	1	1	6	4.4	4.0	3.9	0.939
8	1	2	6	4.2	3.3	3.0	0.912
1	2	1	5	4.5	3.9	4.0	0.936
4	2	1	6	4.2	3.3	3.8	0.927
4	2	2	5	3.0	3.1	2.7	0.932
4	2	4	5	3.1	3.6	3.1	0.920
8	2	4	5	2.7	3.0	2.5	0.919
1	4	2	4	3.2	2.9	2.3	0.932
4	4	4	2	3.1	2.7	2.5	0.907
8	4	1	3	2.5	3.1	2.4	0.909

[a] Crumb Quality: 9 = excellent; 1 = unsatifactory

[b] 5 = Absence of off-flavor; 0 = pronounced off-flavor

[c] Crumb Texture: 5 = smooth and moist; 0 = coarse and dry

[d] Overall Preference: 5 = like very much; 0 = dislike very much

[e] a_w of crumb was determined 24 hours after baking

Staling

Bread staling was followed by the increase in crumb firmess and the increase in starch crystallinity as detected by differential thermal analysis (DSC). As bread stales, the starch reverts from the amorphous form to the more stable, crystalline form. Melting of the crystalline amylopectin occurs at 50-60°C. The endothermic peak area is proportional to the amount of crystalline amylopectin present in the aged bread. Figure 3 shows the DSC scans of a fresh bread and a stale bread stored at 20°C for 14 days. Fresh bread shows no peak at 50°C since the amylopectin is in the amorphous form.

Firming of bread crumb was accelerated by the addition of 10% and 20% sorbitol (Figure 4). The DSC data also shows more starch crystallinity in the breads containing sorbitol compared to the control during the first week of storage at 20°C (Figure 5). However, the control showed more starch crystallinity than the sorbitol bread at day 14. The discrepancy between the firmness and the DSC results indicates the complexity of the staling phenomenon. Factors other than starch retrogradation may also contribute to firming of bread crumb during storage.

Several experiments were conducted to study the effect of sodium stearoyl-2-lactylate (SSL) and shortening on bread staling. For prolonged storage (14 days or longer), 3-9% shortening and/or 0.5-1.5% SSL were not effective in retarding bread staling.

Refinement of Formulation

We observed that browning was a problem with the humectant breads during storage, especially at elevated temperatures. Therefore, an experiment was conducted to determine the effect of sugar (0-6%), malt (0-0.3%) and lactic acid (0-0.3%) concentrations on nonenzymatic browning of the humectant bread made with 8% sorbitol, 2% propylene glycol and 2% glycerol (8-2-2). It was concluded that 3% sugar was the optimum level for minimizing browning while retaining good loaf volume of the bread. Also, addition of 0.15-0.30% lactic acid or 0.1-0.3% malt had little effect on gas production or loaf volume of the bread (see the Annual Report for details). Therefore, malt was completely eliminated and sugar was reduced from 6 to 3% in all subsequent studies to minimize nonenzymatic browning.

pH

Two different types of lactic acid have been used to lower pH of the humectant breads in this study. A lactic acid powder (Purac Powder H) containing 60% lactic acid and 40% calcium lactate was obtained from Patco Products (Kansas City, MO) and a lactic acid solution (85%) was purchased from Sigma Chemical Co. (St. Louis, MO). Because of the buffering effect of calcium lactate, the powder is not as effective as the lactic acid solution in pH reduction (Figure 6). However, the dough handling properties were much better with the lactic acid powder than the solution. Taking into consideration pH and flavor of the bread, 0.3% lactic acid powder was used in the final formulation.

Water Activity Measurement

Moisture content and a_w of bread crumb and bread crust change continuously after baking due to moisture transfer from the crumb to the crust. Therefore, widely variable a_w values may be obtained if the sampling time and location are not well controlled. Figure 7 illustrates the variation of a_w in crumb and crust of the 5-2-2 humectant bread with storage time at 20°C. At day zero, the a_w was 0.94-0.96 for bread crumb and 0.71-0.78 for bread crust. As moisture migrated from the crumb to the crust, a_w of crumb decreased with a corresponding increase in crust a_w.

After 3 weeks of storage, the a_w values of crumb and crust were about 0.92 and 0.88, respectively. Thus, the a_w of the bread would approach an equilibrium value of 0.90. Since the a_w gradually leveled off after one week of storage, it was decided to take the a_w measurement of bread seven (7) days after baking. The crumb samples were taken from the center of the slices that were 1-2 inches from the loaf center. This sampling procedure should help minimize the variation of a_w measurement.

(II) Microbial Challenge Studies

Microbial stability of the bread samples is probably the most important aspect of this project. It has been shown that the lower a_w limit for growth of most molds is 0.80-0.87 (Beuchat, 1981). Since the humectant breads had a pH of 4.8-5.0 and an a_w value of about 0.94-0.95 for fresh crumb, they may be susceptible to microbial spoilage. Therefore, a series of studies were conducted to challenge the bread with Penicillium expansum, Aspergillus niger, Clostridium sporogenes and Staphylococcus aureus.

Molds

Initially, the challenge study was conducted with humectant breads containing 5% sorbitol, 2% propylene glycol and 2% glycerol (5-2-2). No visible growth of A. niger or P. expansum was observed after the breads had been stored for 1,2,4 and 8 weeks at 30°C in retort pouches flushed with CO_2. However, these two molds grew rapidly on regular breads, which served as the positive control.

The minimal a_w values of growth are 0.83 - 0.85 for P. expansum (Beuchat, 1981) and 0.77 for A. niger (Pitt, 1975). These values are substantially below the a_w of the fresh bread crumb (a_w = 0.94). The pH of the bread samples was also too high (4.8 - 4.9) to be inhibitory against the molds. Therefore, the two organisms may be inhibited by the presence of calcium propionate, the humectants, the carbon dioxide, or a combination of these and other factors including a_w and pH. Propylene glycol has been shown to be an effective humectant-antimicrobial agent (Acott et al., 1976). Most likely not any single factor alone was solely responsible for the inhibitory action against the two mold species.

A recent report in Cereal Foods World (Brody, 1985) pointed out that some European bakeries have used carbon dioxide packaging to extend shelf-life of breads. Research conducted by the British Flour Milling Research Association demonstrated that 25% or more carbon dioxide is effective in inhibiting mold growth in baked goods. Therefore, the CO_2 packaging used in this study may play an important role in preventing microbial spoilage of the humectant breads.

Clostridium sporogenes

The vegetative cell count (21-42) and spore count (10-29) of Clostridium sporogenes did not increase with storage time. Although the bread dough was inoculated with about 1000 spores/g of C. sporogenes, only 42 cells and 18 spores per g were detected in the fresh crumb sample located at the center of the loaf. Thus the vast majority of the spores were destroyed during the baking process. The vegetative cells detected probably resulted from spore germination.

Additional challenge studies were conducted using the humectant breads made with the modified formulas (7-1-2 and 6-1.5-2). The results for Clostridium sporogenes are summarized in Table 5. The vegetative cell and spore counts decreased from 46-49 and 13-79 to 0-5 and 2-7, respectively. Also, a slight reduction in crust a_w was observed. The decrease in a_w may indicate a gradual

Table 5. Effect of storage time at 30°C on microbial counts of humectant breads challenged with *Clostridium sporogenes*.[a]

Time	7-1-2[b]				6-1.5-2[b]			
(weeks)	Crust a_w	pH	Veg. Cell (#/g)	Spores (#/g)	Crust a_w	pH	Veg. Cells (#/g)	Spores (#/g)
0	-	-	49	79	-	-	46	13
2	-	-	11	8	-	-	23	13
4	-	-	4	23	-	-	14	2
8	0.923	4.85	7	11	0.926	4.82	2	17
11	0.922	4.75	2	8	0.925	4.77	7	2
16	0.911	4.80	10	4	0.915	4.77	7	2
21	0.913	4.74	0	2	0.913	4.76	5	7

[a] Initial inoculum - 1400/g

[b] % sorbitol - % propylene glycol - % glycerol

dehydration due to the permeability of the film and a small leak.

For a positive control, regular breads containing no humectants were challenged with C. sporogenes and stored in a retort pouch flushed with CO_2. The initial spore count was 130/g and the vegetative cell count was 46/g. During storage at 30°C, the spore count decreased to 8-13/g after 1-3 weeks. However, the vegetative cells multiplied rapidly upon storage. The cell counts/g were 2400 (day 7), 14,300 (day 14) and 14,000 (day 21). This clearly demonstrates the ability of C. sporogenes to grow in bread under favorable conditions.

The minimal a_w for growth is 0.935-0.965 for C. sporogenes (Sperber, 1983). This range is similar to those for C. botulinum (a_w = 0.93-0.94) and C. perfringens (a_w = 0.93-0.95). Although these a_w values are close to that of the bread crumb (a_w 0.93-0.95) the relatively low pH (4.8-4.9) of the humectant breads was inhibitory to the growth of C. sporogenes. While pH (<4.5) or a_w (<0.93) alone could be used to control botulism, inhibition of C. botulinum should be considered in light of the combination of a_w, pH and other factors. Further studies to determine the combined pH and a_w limits for growth and toxin production of C. botulinum in different food systems are necessary.

Staphylococcus aureus

Growth of Staphylococcus aureus on the crust of humectant breads stored at 30°C in CO_2 pouches is shown in Table 6. S. aureus was inhibited in some of the samples. It is not clear why the 6-1.5-2 breads appeared to be more favorable for S. aureus growth than the 7-1-2 breads. Both formulations included 0.3% lactic acid in powder form. Since the bread samples supported the growth of S. aureus, further studies were conducted to evaluate the pH effects.

Humectant breads (7-1-2) were prepared using different concentrations of lactic acid in liquid or powder forms. Loaf volume and pH of the breads decreased with increasing lactic acid concentrations (Table 7 and Figure 8). Mixing time and fermentation time were also affected by the addition of lactic acid. The lactic acid powder contains 60% lactic acid and 40% calcium lactate. Because of the buffering effect of calcium lactate, the powder is not as effective as lactic acid solution in pH reduction. However, the dough handling properties were much better with the lactic acid powder than the solution.

Table 8 shows the effect of lactic acid concentration (powder form) on the growth of S. aureus in humectant breads after one week of storage at 30°C. The preliminary results indicate that 0.25% lactic acid was effective in inhibiting S. aureus when the crust a_w was 0.90-0.91. However, an extended storage study indicated that S. aureus growth was detected in the humectant breads containing 0.3% lactic acid after four weeks of storage at 30°C (Table 9). No growth was observed in the 0.4% lactic acid sample. It should be noted that crust a_w increased continuously after baking, thus creating a more favorable environment for microbial growth. Therefore, adequate storage time should be allowed to evaluate growth of the inoculated organism on the crust. To prevent S. aureus growth, the pH should be reduced to 4.80 or less by incorporating 0.40% or more lactic acid in the humectant breads.

The combined effect of water activity, pH and temperature on growth of S. aureus in brain-heart infusion broth was investigated by Notermans and Heuvelman (1983). At 30°C, S. aureus was inhibited at a_w 0.90 and pH 4.9. This finding is similar to what we observed with the humectant breads.

Increasingly more attention is given to the importance of microbial control by a combination of parameters such as a_w, pH, temperature and inhibitors (Fox and Loncin, 1982; Chang et al., 1983; Webster et al., 1985) This study demonstrates the feasibility of imparting microbial stability to bread by proper control of a_w, pH, packaging and heat treatment.

Table 6. Effect of storage time at 30°C on microbial count of humectant breads challenged with *Staphylococcus aureus*[a].

Time	7-1-2[b]			6-1.5-2[b]		
(weeks)	Crust a_w	pH	Count	Crust a_w	pH	Count
2	-	-	1600	-	-	$5.7x10^5$
			0			$7.8x10^5$
4	-	-	$7.6x10^3$	-	-	$5.2x10^6$
			0			$8.6x10^6$
8	0.917	4.97	400	0.918	4.92	$8.8x10^5$
	0.920	4.96	200	0.922	4.94	400
11	0.914	4.75	0	0.914	4.71	$1.32x10^6$
	0.916	4.75	0	0.918	4.72	$8.2x10^5$
16	0.919	4.80	$6.8x10^6$	0.908	4.81	$1.5x10^6$
	0.917	4.80	$1.3x10^5$	0.908	4.83	0

[a] Initial inoculum - 10^4 CFU on crust surface (1.25 in. diameter circle)

[b] % sorbitol - % propylene glycol - % glycerol

Table 7. Effect of liquid and powdered lactic acid on baking properties of humectant breads containing 7% sorbitol, 1% propylene glycol and 2% glycerol (7-1-2).

Sample	Lactic Acid	H_2O Abs. (%)	Mix Time (min:sec)	Ferment Time(min)	Loaf wt.(g)	Loaf Vol.(c.c)	pH
Control	-	64.4	2:30	136	152.3	983	5.26
7-1-2	-	58.4	4:00	144	163.1	970	5.49
0.3%	Powder	58.4	4:10	168	162.0	945	4.91
0.5%	Powder	58.4	4:22	201	162.6	913	4.60
0.6%	Powder	58.4	4:46	212	160.8	873	4.51
0.2%	Liq.	58.4	4:00	156	163.6	953	4.94
0.3%	Liq.	58.4	4:22	170	162.6	895	4.78
0.5%	Liq.	58.4	5:02	198	161.5	890	4.51

Table 8. Effect of lactic acid (powder form) on survival of <u>Staphylococcus aureus</u> in a CO_2 atmosphere at 30°C in humectant breads containing 7% sorbitol, 1% propylene glycol and 2% glycerol.

Lactic		Crust a_w		Staph. Count	
Acid (%)	pH	Day 2	Day 7	Day 2	Day 7
0[a]	5.48	0.899	0.918	$5.7x10^4$ $3.1x10^4$	TNTC
0.25	5.04	0.902	0.909	$1.2x10^4$ $1.8x10^4$	100
0.30	4.97	0.898	0.914	$1.3x10^4$ $1.6x10^4$	<100
0.35	4.87	0.898	0.910	$1.2x10^4$ $1.2x10^4$	<100
0.425	4.76	0.892	0.914	$0.9x10^4$ $0.7x10^4$	<100
0.50	4.71	0.900	0.915	$0.7x10^4$ $0.65x10^4$	<100

[a] Initial count at day zero = $1.4x10^4$ on crust surface (1.25 in. diameter circle)

Table 9. Effect of lactic acid (powder form) on survival of *Staphylococcus aureus* at 30°C on humectant breads containing 7% sorbitol, 1% propylene glycol and 2% glycerol.

Time	0.3% lactic			0.4% lactic		
(weeks)	pH	a_w	Count	pH	a_w	Count
0[a]	5.00	0.947	1.28×10^4	4.87	0.953	1.31×10^4
	5.01	0.954	1.40×10^4	4.87	0.948	1.39×10^4
2	4.84	0.902	0	4.72	0.905	50
	4.84	0.896	0	4.73	0.903	0
4	4.81	0.911	0	4.69	0.907	0
	4.81	0.929	8.8×10^4	4.67	0.916	0

[a] pH and crust a_w measured at day 5

Conclusions

(1) Penicillium expansum and Aspergillus niger did not grow in the humectant breads containing 5% sorbitol, 2% propylene glycol and 2% glycerol (5-2-2) when packaged in a CO_2 atmosphere.
(2) The 7-1-2 and 6-1.5-2 humectant breads did not support the growth of Clostridium sporogenes during 21-weeks storage at 30°C, although rapid growth was observed in regular breads stored under the same conditions after 1-2 weeks.
(3) To prevent S. aureus growth on bread crust, pH of the humectant breads should be reduced to 4.8 or less with lactic acid.

(III.) Storage stability Studies

An extensive storage stability test was conducted using the 7-1-2 and 6-1.5-2 humectant breads. The two formulas were selected based on the information obtained from previous experiments regarding acceptability and microbial stability of the samples. The breads were packaged in retort pouches in air or CO_2 and stored at 20,30 or 45°C. Samples were analyzed periodically for mold, aerobic, and anaerobic counts, moisture content, a_w, firmness, pH and color. Also, a number of sensory attributes were evaluated by a trained taste panel. A complete set of the data for the physical and sensory properties is given in Appendix E-I.

Microbial Stability

No visible mold growth was detected in any of the samples throughout the 170 day storage period (Table 10). The aerobic counts of the 7-1-2 samples did not exceed 1500/g. However, the 6-1.5-2 sample showed excessively high aerobic counts at days 113 and 170. Anaerobic counts of the breads remained low until day 170 when they became too numerous to count. Thus the anaerobes in the humectant breads grew between day 113 and day 170. It is not clear why such a long lag period was observed for the growth of the anaerobes. It is possible that sufficient oxygen was present to inhibit them and it took this long for the aerobes to deplete it. Based on the microbiological data, the shelf lives were limited to about 113 days for the 7-1-2 bread, and 85 days for the 6-1.5-2 bread. Both formulations included only 0.3% powdered lactic acid. As pointed out in the "Microbial Challenge Study" section, lactic acid concentration should be increased to 0.4% or higher to inhibit S. aureus. Therefore neither formulation offered adequate protection against microbial spoilage during long term storage at ambient temperature.

Relatively little information is available in the literature regarding microbial stability of bread packaged in carbon dioxide. A storage study of English style crumpets packaged in CO_2 showed a substantial increase in aerobic plate count from 3×10^3 to 21×10^7 after 19 days at 30° C (Smith et al., 1983). The pH of the product was 6.5, considerably higher than that of the humectant bread. The short shelf life of the CO_2-packaged crumpets illustrates the importance of pH and a_w reduction in controlling microbial growth in the humectant bread.

Moisture and Water Activity

Changes in moisture content of crumb and crust of the two humectant breads (7-1-2 and 6-1.5-2) during storage are shown in Figures 8-11 and Table 11. The crust moisture increased rapidly during the first two weeks of storage and leveled off at about 36% (dry basis) for the 7-1-2 sample and at about 39% (dry basis) for the 6-1.5-2 sample. Conversely, crumb moisture decreased rapidly during the first two weeks and leveled off at about 44% (dry basis) for the

Table 10. Microbial counts of humectant breads stored at 30°C

Days	7-1-2[a]			6-1.5-2[a]		
	Mold	Aerobic	Anaerobic	Mold	Aerobic	Anaerobic
	(CFU/g)			(CFU/g)		
0	390	5	0	0	0	0
6	0	5	0	0	0	0
32	0	0	0	0	43	0
66	0	35	0	0	1300	0
85	0	1500	2	0	0	0
113	0	260	0	0	10^4	0
170[b]	0	85	TNTC[c]	0	1.5×10^7	TNTC[c]

[a] % sorbitol - % propylene glycol - % glycerol

[b] Packed in CO_2

[c] Too numerous to count

Table 11. Statistical comparison of the storage data of the two humectant breads.

Parameters	7-1-2[c]	6-1.5-2[c]
Moisture (% d.b.)		
Crumb	46.0[a]	49.3[b]
Crust	35.5[a]	37.0[b]
a_w		
Crumb	0.927[a]	0.932[a]
Crust	0.915[a]	0.921[a]
pH	4.93[a]	5.01[b]
Firmness (g)		
20° CO_2	1107[a]	970[b]
30° CO_2	1023[a]	880[b]
30° Air	880[a]	736[b]
45° CO_2	-	1015

[a,b] Values on the same row with different letters are significantly different ($p < 0.05$).

[c] % sorbitol - % propylene glycol - % glycerol.

7-1-2 sample and 48% (dry basis) for the 6-1.5-2 sample. Based on the moisture data, the retort pouch was an excellent moisture barrier for the packaged breads.

Water activity changes in crumb and crust of the two humectant breads during storage are shown in Figures 12-15 and Table 11. The a_w values were more scattered than the moisture values. This illustrates the difficulty in obtaining precise and accurate a_w measurements. For the 7-1-2 humectant bread, the crust a_w varied between 0.905 - 0.925 and the crumb a_w was 0.920 - 0.930 after 10 days of storage. For the 6-1.5-2 bread, the crust a_w and crumb a_w were 0.910 - 0.930 and 0.920 - 0.935, respectively. Thus, a_w of the bread samples did not exceed 0.935 after 10 days of storage.

Statistical analysis of the moisture and a_w data shows no significant difference in a_w between the 7-1-2 and the 6-1.5-2 humectant breads (Table 11). However, the 6-1.5-2 breads had significantly higher crumb and crust moisture contents than those of the 7-1-2 breads. This could be associated with the growth of aerobic organisms in the 6-1.5-2 samples but not in the 7-1-2 samples after 113 days of storage at 30°C.

pH

The pH of the bread crumb decreased with increasing storage time (Table 12, Figures 16-19). Similar pH reduction in CO_2-packaged English style crumpets during storage at 20°C was observed by Smith et al. (1983). They ascribed the decrease in pH to absorption of CO_2 in the package. Products of nonenzymatic browning could also contribute to the decrease in pH during storage. The pH values of 4.93 and 5.01 are higher than desired. As indicated in the section on microbial challenge studies, the pH should be 4.8 or less in order to improve microbial stability.

Firmness

Firmness of bread crumb as measured by the Fudoh Rheometer in g force increased with increasing storage time (Figures 20-22). The 7-1-2 bread had firmer crumb than the 6-1.5-2 bread. This is probably due to the higher moisture content of the 6-1.5-2 samples. Firming of bread crumb occurred more rapidly in breads stored at 45°C than those stored at 30°C. This is somewhat surprising since lower storage temperature is known to accelerate firming of bread crumb. Perhaps mechanisms other than starch retrogradation contributed to crumb firming at high temperature during prolonged storage.

It should be pointed out that in the packaging of the bread total displacement of the air atmosphere by CO_2 was not obtained. Analysis of the CO_2 content indicated an average value of 63.7% with a range of 44.8-77%. Comparison of the firmness readings for breads stored in CO_2 atmosphere versus air (Figure 20) indicates that firming is more rapid in the presence of CO_2. This is diametrically opposed to the observations of Knorr and Tomlins (1985) who showed that compressibility of breads stored for 1-2 weeks increased much more slowly in the presence of CO_2 atmospheres as opposed to air or nitrogen. This apparent discrepancy may be due to the differences in storage time in the two studies (2 weeks vs. 6 months).

Although SSL and additional shortening were added to the humectant bread, their effect on bread firmness was probably minimal after the first two weeks of storage.

Color

The Hunter L values for the crumb and crust of the stored breads are presented in Tables 13 and 14. Very little change in the lightness value was

Table 12. Statistical analysis of the storage data of the two humectant breads showing the effect of time on moisture, a_w, pH and firmness.

	Time (Day)									
	1	8	15	22	43	65	85	113	141	169
Moisture (% db. crumb)	57.6^{a}	48.3^{bc}	46.0^{cd}	48.6^{b}	46.1^{cd}	45.5^{d}	45.7^{cd}	45.6^{d}	46.9^{bcd}	45.4^{c}
a_w (crumb)	0.949^{a}	0.939^{b}	0.931^{c}	0.928^{cd}	0.920^{e}	0.925^{c}	0.931^{c}	0.930^{c}	0.928^{cd}	0.91[illegible]
a_w (crust)	--	--	0.919^{b}	0.918^{bc}	0.908^{d}	0.924^{a}	0.927^{a}	0.911^{d}	0.925^{a}	0.91[illegible]
pH	5.00	5.05	5.12	5.04	5.06	5.01	4.89	4.85	4.88	4.8[illegible]
Firmness (g)	357^{a}	539^{b}	783^{c}	890^{d}	1135^{d}	1146^{d}	1160^{d}	1206^{d}	1141^{d}	114[illegible]

a,b,c,d, Values in the same row with different letters are significantly different ($p < 0.05$).

Table 13. Hunter L values of the crumb of stored bread.

Time (weeks)

Sample	0.14	1	2	3	6	9	12	16	20
7:1:2 20° CO_2	73.2	-	-	71.4	73.9		74.7	71.0	73.2
6:1.5:2 20° CO_2	74.9	-	-	74.1	75.1		75.4	68.9	74.3
7:1:2 30° CO_2	-	73.4	72.3	73.5	72.5	72.0	71.8	66.0	71.6
6:1.5:2 30° CO_2	-	74.3	73.7	74.5	74.6	73.3	74.0	66.2	73.6
7:1:2 30° Air	-	73.4	73.6	74.5	74.4	73.8	72.7	71.5	71.4
6:1.5:2 30° Air	-	74.9	74.0	74.7	74.4	74.5	74.5	72.7	73.5
7:1:2 45° CO_2	-	72.0	71.9	69.8	67.7	60.2	-	-	-
6:1.5:2 45° CO_2	-	73.5	73.4	73.9	71.0	67.8	-	-	-

Table 14. Hunter L values of the crust of stored bread.

	Time (weeks)									
Sample	0.14	1	2	3	6	9	12	16	20	24
7:1:2 20° CO_2	39.6	-	-	31.2	29.2	-	36.4	31.6	27.9	30.4
6:1.5:2 20° CO_2	34.5	-	-	33.3	34.5	-	34.2	27.7	27.8	32.3
7:1:2 30° CO_2	-	32.6	27.5	30.8	29.5	29.1	29.3	29.9	29.2	32.6
6:1.5:2 30° CO_2	-	33.5	29.7	29.8	31.9	34.7	29.1	25.2	34.0	28.6
7:1:2 30° Air	-	30.6	31.2	31.9	39.8	31.6	28.3	30.5	30.4	32.1
6:1.5:2 30° Air	-	31.8	30.7	29.4	29.6	29.4	29.9	24.8	26.8	29.4
7:1:2 45° CO_2	-	27.3	28.0	26.3	26.0	26.8	-	-	-	-
6:1.5:2 45° CO_2	-	30.3	25.7	30.0	28.9	35.8	-	-	-	-

observed for the bread stored at 20 and 30°C. However, at 45°C there was a dramatic decrease in the lightness value for the crumb indicating a rapid state of browning. Not as dramatic a trend was observed in the crust stored at 45°C (Table 14).

Sensory Evaluation

A total of 25 sensory attributes of the humectant breads were evaluated as a function of storage time (Tables 15-17, Figures 23-72, Appendix F). As expected, crumb and crust color increased during storage (Figures 23, 24, 48, 49, Table 15). Browning was accelerated at 45°C. The control stored at -20°C showed no significant changes in crumb or crust color during storage. Carbon dioxide has no significant effect on browning.

In general, the aroma values showed significant increases during storage and this was reflected in the overall aroma intensity (Tables 15-17, Figures 25-29, 50-54). The only aroma that did not significantly change during storage was the sour aroma. Of interest is the observation that the caramel character did not begin to appear until 36 days of storage and then it increased dramatically by the end of 78 days. Among all of the aroma attributes, rancid aroma showed the most pronounced changes (Figures 29, 54). The bread stored at 45°C became highly rancid after two weeks. The overall aroma intensity of the samples stored in CO_2 was lower than those stored in air at 30°C during the first five weeks (Table 16). It should be noted that yeasty aroma, an indication of freshness of bread, decreased with storage time (Figures 26, 50).

Textural changes in bread during storage are shown in Tables 15-17, and Figures 30-33 and 50-58. Firmness, dryness, crumbliness and springiness increased with storage time. These changes generally occurred more rapidly at 45°C than at 20 and 30°C. This is contrary to the general belief that rate of bread staling decreases with increasing temperature. Perhaps mechanisms other than starch retrogradation are involved in bread staling at high storage temperature. Similar changes occurred in mouthfeel (Figures 34-37, 59-62), which can also be considered textural in nature. Graininess and dryness showed the most dramatic changes during storage. Gumminess and rate of hydration did show a tendency to decrease in values but not as dramatically as the perceived increase in graininess. Carbon dioxide had no significant effect on textural changes in bread during storage at 30°C (Tables 16 and 17).

Among all of the flavor attributes, rancid and caramel flavors showed the most dramatic changes during storage (Tables 15-17, Figures 38-46, 63-71). Sourness and bitterness increased with storage time, while yeasty flavor, an indicator of freshness, decreased. Sweetness showed little change during storage. As expected, there was a significant interaction between temperature and sensory properties.

All of the changes in sensory properties in bread during storage are reflected in the overall preference (Figures 47 and 72). The control (-20°C) remained unchanged during the storage period, while preference of the 45°C sample decreased markedly with storage time. No beneficial effect of CO_2 storage was observed regarding sensory preference of the bread.

In addition to the trained taste panel tests, a preference test was also conducted after five weeks storage of the bread (Table 18) using an untrained panel. No significant difference was observed between the 7-1-2 and the 6-1.5-2 samples. Preference score decreased with increasing temperature. The humectant breads stored at 30°C were rated as "neither like nor dislike", while the 45°C samples were unacceptable. It should be pointed out that bread as a food normally would not generate a high preference score since bread is usually used as a carrier.

Table 15. Statistical analysis of the sensory data during storage of two humectant breads stored at -20°C, 20°C (CO_2), 30°C (CO_2) and 30°C (air).

Sensory Attributes	Days						Formula	
	8	15	36	78	120	168	6-1.5-2	7-1-2
Crumb color	18.4^{a}	18.9^{a}	19.0^{a}	20.7^{ab}	23.7^{bc}	24.3^{c}	19.5^{a}	22.2^{b}
Aroma								
overall intensity	25.8^{a}	24.9^{a}	28.2^{ab}	28.2^{ab}	31.2^{bc}	32.8^{c}	N.D.	
acrid aroma	6.5^{a}	6.1^{a}	6.4^{a}	8.9^{ab}	10.7^{b}	10.0^{b}	N.D.	
sour aroma	6.5^{ab}	6.2^{a}	5.8^{a}	5.5^{a}	7.9^{ab}	8.8^{b}	6.1^{a}	7.5^{b}
caramel aroma	-	-	$.9^{a}$	9.7^{b}	10.7^{b}	11.9^{b}	N.D.	
rancid aroma	3.2^{a}	4.8^{a}	5.7^{ab}	9.1^{c}	8.8^{bc}	8.4^{bc}	N.D.	
Texture								
dryness	16.3^{a}	19.1^{ab}	22.8^{b}	27.6^{c}	27.0^{c}	27.7^{c}	21.5^{a}	25.2^{b}
springiness	26.0^{a}	30.8^{b}	31.8^{b}	34.9^{b}	33.8^{b}	33.6^{b}	30.3	33.2
crumbliness	11.7^{a}	17.0^{b}	20.2^{bc}	21.1^{bcd}	22.2^{cd}	25.4^{d}	N.D.	

Table 15 (cont.)

	Days						Formula	
Sensory Attributes	8	15	36	78	120	168	6-1.5-2	7-1-2
Mouthfeel								
gumminess	25.6^a	25.0^a	24.4^a	29.8^b	26.2^a	24.4^a	N.D.	
graininess	6.5^a	7.8^a	12.2^b	13.2^b	15.3^b	15.7^b	N.D.	
rate of hydration	30.7^b	30.8^b	27.8^{ab}	27.5^{ab}	29.9^{ab}	25.6^a	30.8^b	26.0^a
Flavor								
sweet	16.1^a	15.2^a	17.2^{ab}	18.6^b	18.5^b	16.5^{ab}	N.D.	
sour	4.1^a	4.1^a	5.1^a	6.1^{ab}	8.4^{bc}	9.5^c	N.D.	
bitter	3.6^a	5.0^{abc}	3.8^{ab}	4.1^{ab}	5.5^{bc}	6.1^c	N.D.	
yeasty	N.D.					N.D.		
caramel	5.8^a	6.2^a	6.1^a	11.5^b	13.5^b	10.7^b	N.D.	
rancid	4.6^a	6.3^{ab}	8.4^{bc}	10.8^c	11.1^c	11.4^c	N.D.	

Values in the same row with different letters are significantly different at 5% level.

N.D. = Not determined

Table 16. Statistical analysis of the sensory data for two humectant breads stored for five weeks at -20, 30 and 45°C.

Sensory Attributes	Storage Conditions -20(CO_2)	30(Air)	30(CO_2)	45(CO_2)
Crust browness	significant interaction			
Crumb color	16.4^{a}	19.8^{b}	20.1^{b}	28.1^{c}
Aroma				
overall intensity	24.2^{a}	28.0^{b}	26.6^{a}	31.3^{c}
yeasty aroma	13.3^{a}	10.0^{b}	10.7^{b}	9.5^{b}
acrid aroma	4.8^{a}	7.9^{b}	6.3^{ab}	8.0^{b}
sour aroma	4.2^{a}	7.4^{b}	6.8^{ab}	8.1^{b}
caramel aroma	not tested these weeks			
rancid aroma	2.5^{a}	5.9^{b}	5.3^{ab}	9.6^{c}
Texture				
dryness	11.1^{a}	22.7^{b}	24.3^{b}	29.3^{c}
firmness	significant interaction			
springiness	21.1^{a}	34.5^{bc}	33.0^{b}	38.2^{c}
crumbliness	10.1^{a}	18.7^{b}	20.2^{b}	22.1^{b}
Mouthfeel				
dryness	12.3^{a}	26.0^{b}	26.0^{b}	32.7^{c}
gumminess	28.8^{a}	22.7^{b}	23.7^{b}	20.9^{b}
graininess	6.2^{a}	9.4^{ab}	10.9^{b}	12.1^{b}
rate of hydration	36.9^{b}	27.2^{a}	26.1^{a}	24.9^{a}

Table 16 (cont.)

Sensory Attributes	Storage Conditions			
	$-20(CO_2)$	30(Air)	$30(CO_2)$	$45(CO_2)$
Flavor				
sweet	N.D			
sour	3.6^{a}	5.5^{ab}	4.2^{ab}	6.4^{b}
bitter	N.D			
yeasty	11.1^{b}	7.1^{a}	8.4^{a}	7.1^{a}
caramel	5.7^{a}	6.3^{ab}	6.1^{ab}	8.8^{b}
cardboard/rancid	3.1^{a}	7.7^{b}	8.5^{b}	12.6^{c}
Crust flavor				
burnt		significant interaction		
bitter	N.D			
sweet	N.D			

Values in the same row with different letters are significantly different at 5% level.

N.D. = Not determined

Table 17. Statistical analysis of the sensory data for two humectant breads stored for 24 weeks at -20 and 30°C

Sensory Attributes	Storage Conditions		
	$-20(CO_2)$	30(AIR)	$30(CO_2)$
Crust browness	significant interaction		
Crumb color	15.6^a	22.8^b	24.1^b
Aroma			
overall intensity	24.6^a	31.0^b	30.0^b
yeasty aroma	14.2^b	9.3^a	9.6^a
acrid aroma	4.3^a	10.4^b	9.5^b
sour aroma	3.9^a	8.7^b	1.7^b
caramel aroma	3.8^a	9.8^b	11.1^b
rancid aroma	2.5^a	9.1^b	8.4^b
Texture			
dryness	17.7^a	27.9^b	30.5^b
firmness	significant interaction		
springiness	22.1^a	35.7^b	37.5^b
crumbliness	10.4^a	23.5^b	24.8^b
Mouthfeel			
dryness	significant interaction		
gumminess	31.5^b	23.6^a	22.4^a
graininess	6.3^a	14.2^b	14.6^b
rate of hydration	37.5^b	24.8^a	24.3^a

Table 17 (cont.)

	Storage Conditions		
Sensory Attributes	-20(CO_2)	30(AIR)	30(CO_2)
Flavor			
sweet	N.D		
sour	4.5^a	7.2^b	7.0^b
bitter	2.9^a	5.9^b	5.2^b
yeasty	11.6^b	6.9^b	7.3^b
caramel	5.7^a	10.6^b	10.5^b
rancid	3.6^a	11.7^b	10.9^b
Crust flavor			
burnt	10.6^b	9.0^a	10.1^{ab}
bitter	N.D		

Values in the same row with different letters are significantly different ($p<0.05$).
N.D. = Not determined

Table 18. Sensory preference of the humectant breads after five weeks of storage

Sample	Storage Conditions	Preference Score	Nine-Point Equivalent
6-1.5-2	45°C	21.3[a]	3.2
7-1-2	45°C	22.3[a]	3.3
7-1-2	30°C	29.7[b]	4.5
6-1.5-2	30°C	30.0[b]	4.5
6-1.5-2	-20°C	37.5[c]	5.6
7-1-2	-20°C	40.5[c]	6.1

Means in the same column with different letters are significantly different at 5% level.

Conclusions

1. No visible mold growth was detected in the 7-1-2 or 6-1.5-2 samples throughout the 170-day storage period.
2. Anaerobic growth occurred between 113 and 170 days at 30°C. Aerobic count also increased markedly in the 6-1.5-2 sample by 113 days.
3. Lactic acid (powder) concentration should be increased from 0.3 to 0.4%.
4. The retort pouch provided an excellent barrier for the packaged bread. No moisture loss occurred during the storage period. Water activity of the bread crumb and crust stabilized at about 0.92-0.93 after extended storage.
5. Storage of the humectant bread resulted in an increase in crumb firmness, an increase in browning and a decrease in pH. These changes were generally accelerated at 45°C compared to 20 and 30°C.
6. Most aroma values of the humectant breads increased with storage time. Rancid aroma showed the most dramatic change. Yeasty aroma, an indicator of freshness, decreased with storage time.
7. Firmness, dryness, crumbliness and springiness of the humectant breads increased with storage time. These changes generally occurred more rapidly at 45°C than at 20 and 30°C.
8. Rancid and caramel flavors of the humectant bread increased markedly during storage.
9. The control (-20°C) showed no significant changes in physical or sensory properties throughout the storage period.
10. Carbon dioxide storage did not improve the sensory acceptability of humectant bread during prolonged storage.

(IV.) Refinement of Formula

Effect of Shortening and Oil

Since rancidity was a major problem in bread staling during storage, an experiment was conducted to evaluate the effect of fat content on bread baking properties. A reduction in fat content was accompanied by an increase in water absorption in bread dough (Table 19). From the standpoint of microbial stability, a high fat content (e.g. 10%) is preferred.

In an attempt to alleviate the rancidity problem, an oil that is highly stable against lipid oxidation (Durkex 500) was used in place of the Crisco shortening (Table 20). Compared to the humectant bread (7-1-2) containing 10% shortening, the oil sample had a higher specific volume and a higher crumb water activity. After 10 days of storage at 45°C, a_w of the crumb and crust of the two samples was about 0.92, indicating an equilibration between the crumb and the crust.

In general, there were no significant differences between the oil and the shortening samples stored for 10 days at 45°C (Table 21). The only exception was that sourness was more pronounced in the shortening sample. The changes in sensory properties were similar to those reported in Tables 15-17.

Sponge vs. Straight Dough

The humectant breads (7-1-2) prepared by the straight dough and the sponge dough methods were similar in loaf volume, crust color, crumb moisture and water activity (Table 22). However, pH of the sponge dough bread (5.0) was lower than that of the straight dough bread (5.2). Thus the sponge dough method is preferred for the production of the humectant breads.

Table 19. Effect of fat content on baking properties of humectant breeds containing 7% sorbitol, 1% propylene glycol, 2% glycerol and 0.4% lactic acid.

Formula (% Fat)	H_2O Abs. (%)	Mix Time (min:sec)	Ferm. Time(min)	Proof Time(min)	Loaf Wt. (g)	Loaf Vol. (cc)	Crumb[a] Grain
Control	64.5	2:30	94	46	150.7	985	7
3	60.4	3:50	100	82	158.2	896	7
6	59.4	4:05	100	88	159.8	912	7
10	58.4	4:00	100	98	162.6	921	7

[a] Excellent = 9, Poor = 1

Table 20. Effect of shortening and a high stability oil (Durkex 500) on baking properties, firmness and a_w of humectant breads containing 7% sorbitol, 1% propylene glycol and 2% glycerol.

		H_2O Abs.	Mix Time	Ferm/Proof	Loaf wt.	Loaf Vol.	Firmness (g)		a_w (Day 7)	
Fat	(%)	(%)	(min:sec)	Time(min)	(g)	(c.c.)	Day 1	Day 7	Crust	Crumb
Control	(3%)	64.5	2:30	133	150.0	983	177±20	-	-	-
Short.	(10%)	58.4	4:09	190	162.0	883	201±33	616±106	0.890	0.909
Oil	(10%)	59.4	4:13	192	160.8	903	148±16	726±81	0.896	0.930
Oil	(10%)	58.4	4:00	185	159.9	903	160±10	604±76	0.898	0.924
Oil	(6%)	60.4	3:32	186	155.8	898	127±18	636±78	0.904	0.929
Oil	(3%)	61.4	2:30	133	150.0	871	129±23	636±90	0.904	0.925

Table 21. Sensory properties of humectant breads (7-1-2) containing 10% shortening or a high stability oil (Durkex 500) after ten days of storage.

	Sensory Score			
Attributes	Oil (-20°C)	Shortening (-20°C)	Oil (45°C)	Shortening (45°C)
Crumb Color	9.7[a]	10.0[a]	18.7[b]	18.7[b]
Overall Aroma Intensity	25.3[ab]	23.1[a]	31.7[bc]	34.2[b]
Yeasty Aroma	15.7[b]	15.7[b]	9.7[a]	9.0[a]
Rancid Aroma	1.7[a]	2.4[a]	13.2[b]	16.1[b]
Dryness	8.9[a]	8.2[a]	31.0[b]	33.4[b]
Firmness	7.5[a]	8.0[a]	36.1[b]	36.2[b]
Crumbliness	7.5[a]	7.5[a]	15.7[b]	25.5[b]
Sour Flavor	3.2[a]	6.4[ab]	8.3[b]	14.2[c]
Caramel Flavor	4.2[a]	4.0[a]	14.3[b]	8.9[ab]
Rancid Flavor	4.7[a]	5.0[a]	17.6[b]	15.1[b]
Preference	30.9[b]	33.1[b]	18.9[a]	17.5[a]

Values in the same row with different letters are significantly different at 5% level.

Table 22. Baking properties and other characteristics of humectant breads made by the straight dough and sponge dough methods.

Sample[a]	Yeast (%)	H_2O Abs. (%)	Mix Time (min:sec) sponge	Mix Time (min:sec) dough	Ferm. Time (min)	Proof Time (min)	Lf wt. (g)	Lf Vol. (cc)	Crust L[b]	pH	Crumb a_w	Moisture (% d.b.)
Straight (control)	5.25	64.4	-	2:30	90	40	151.2	983	52.4	5.41	0.964	57.3
Sponge (control)	2.63	64.4	1:30	2:00	270/15	45	143.6	1015	48.9	5.05	0.956	54.0
Straight (7-1-2)	5.25	58.4	-	4:10	90	62	163.3	960	43.8	5.18	0.941	43.7
Sponge (7-1-2)	2.63	58.4	1:30	3:30	270/15	103	155.1	898	42.3	5.03	0.927	42.9
Sponge (7-1-2)	5.25	58.4	1:30	3:30	270/15	71	157.6	975	43.8	5.04	0.934	45.3

[a] 7-1-2 = 7% sorbitol, 1% propylene glycol, 2% glycerol

[b] Hunter L value

CONCLUSIONS

1. Dough mixing properties were affected by the addition of sorbitol, glycerol and propylene glycol. Propylene glycol was detrimental to dough properties even at low concentrations (2-5%).

2. Loaf volume and water activity (a_w) of bread decreased with increasing humectant concentrations. Compared to sorbitol at the same a_w, glycerol was more detrimental to bread quality.

3. The a_w of crust was lower than the crumb and required time to reach equilibrium.

4. Staling seemed to be accelerated by increased concentration of humectants, although at 14 days no difference was observed between 10% sorbitol and the control. Shortening (3-9%) and sodium stearyl-2-lactylate (0.5-1.5%) did not retard staling.

5. Removing malt from the formula and reducing the sucrose content to 3% markedly reduced the tendency to browning.

6. Lactic acid powder (Purac Powder H) gave better dough handling properties than lactic acid solution although more was needed to obtain the same decrease in pH.

7. Based on a_w, loaf volume and sensory evaluation data, a humectant bread containing 7% sorbitol, 1% propylene glycol and 2% glycerol (7-1-2) was selected for further study.

8. Mold growth in bread can be inhibited by the addition of humectants and lactic acid, and by packaging in a partial CO_2 atmosphere.

9. The 7-1-2 and 6-1.5-2 humectant breads did not support the growth of *C. sporogenes* at 30°C.

10. *S. aureus* grew readily in the 6-1.5-2 bread, while results were variable with the 7-1-2 bread. To prevent *S. aureus* growth, pH of the humectant bread should be decreased to 4.8 or less.

11. An extended storage stability test (170 days) showed no visible mold growth in the 7-1-2 and 6-1.5-2 breads. However, anaerobic growth occurred between days 113 and 170 at 30°C. It is recommended that the lactic acid concentration be increased from 0.3 to 0.4%.

12. Major changes in texture, color, pH and sensory properties of the humectant breads were observed during storage. The breads showed an increase in browning, firmness, dryness, crumbliness, rancid flavor and rancid aroma. These changes were accompanied by a decrease in pH and overall preference score. There was a significant interaction between these changes and temperature.

13. Carbon dioxide storage did not improve the overall sensory acceptability of the breads.

14. Replacing the shortening in the formula with a high stability oil (Durkex) had no significant effect on sensory properties of the humectant bread stored at 45°C for ten days.

15. Humectant bread made with the sponge dough method had a lower pH than that made with the straight dough method.

RECOMMENDATIONS

1. The bread formula should include 7% sorbitol, 1% propylene glycol and 2% glycerol, or a combination of humectants that results in a final a_w of 0.92-0.93 or less in the crumb.

2. The pH of the bread should be reducecd to 4.8 or less by addition of more lactic acid powder.

3. The bread should be packaged in a hermetically sealed container such as a retort pouch or a metal can with a partial CO_2 atmosphere.

4. The bread should be prepared by the sponge dough method.

5. To avoid post-contamination, the bread may be baked inside the container and sealed after baking.

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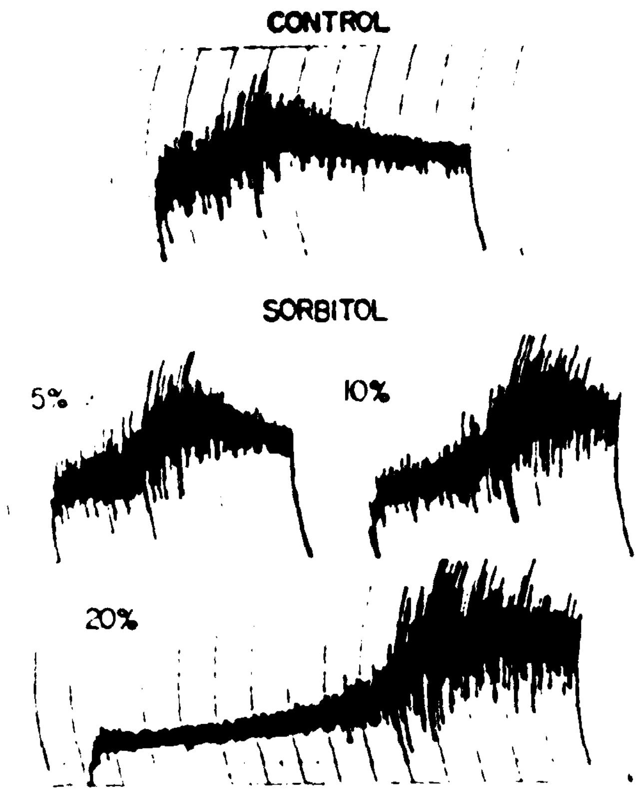

Figure 1. Mixograms of wheat flour doughs containing 0, 5, 10 and 20% sorbitol.

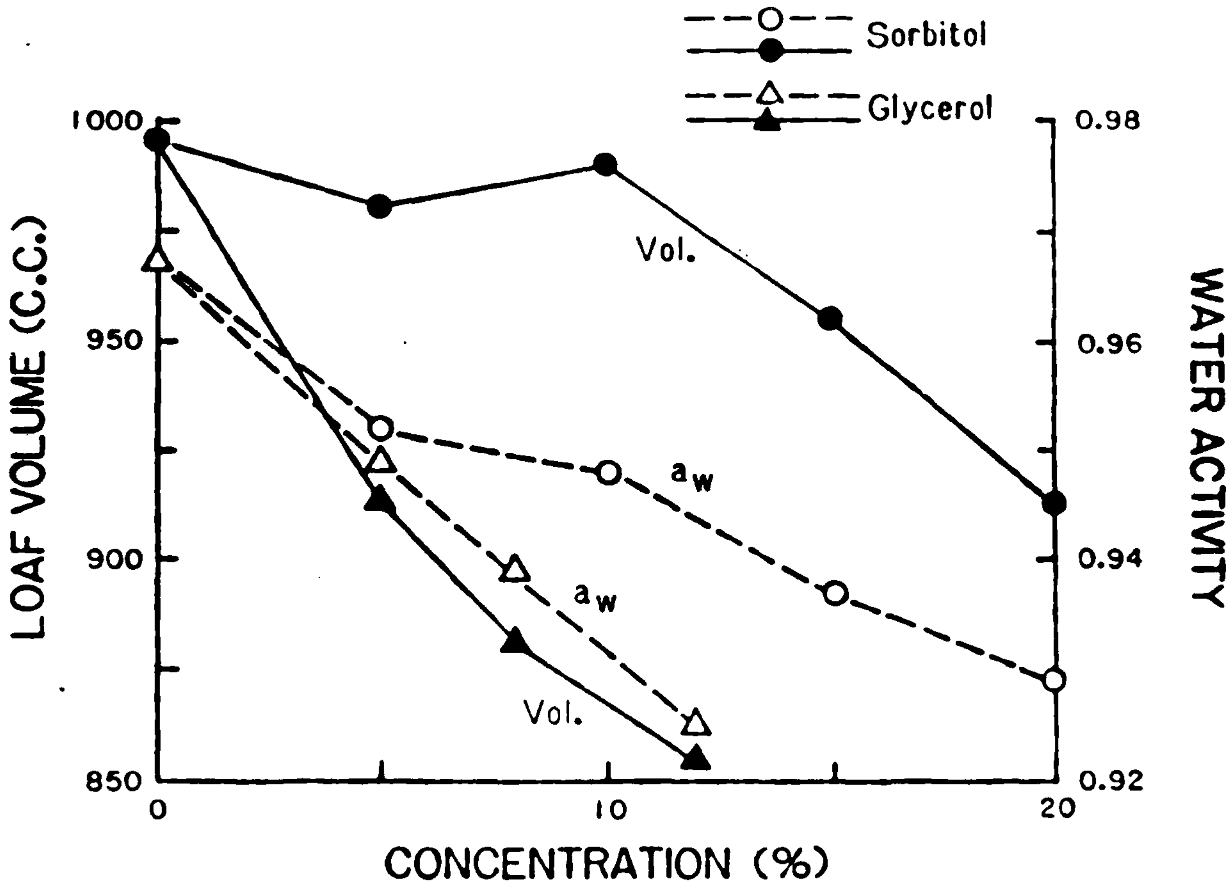

Figure 2. Effect of sorbitol and glycerol on loaf volume and water activity of bread.

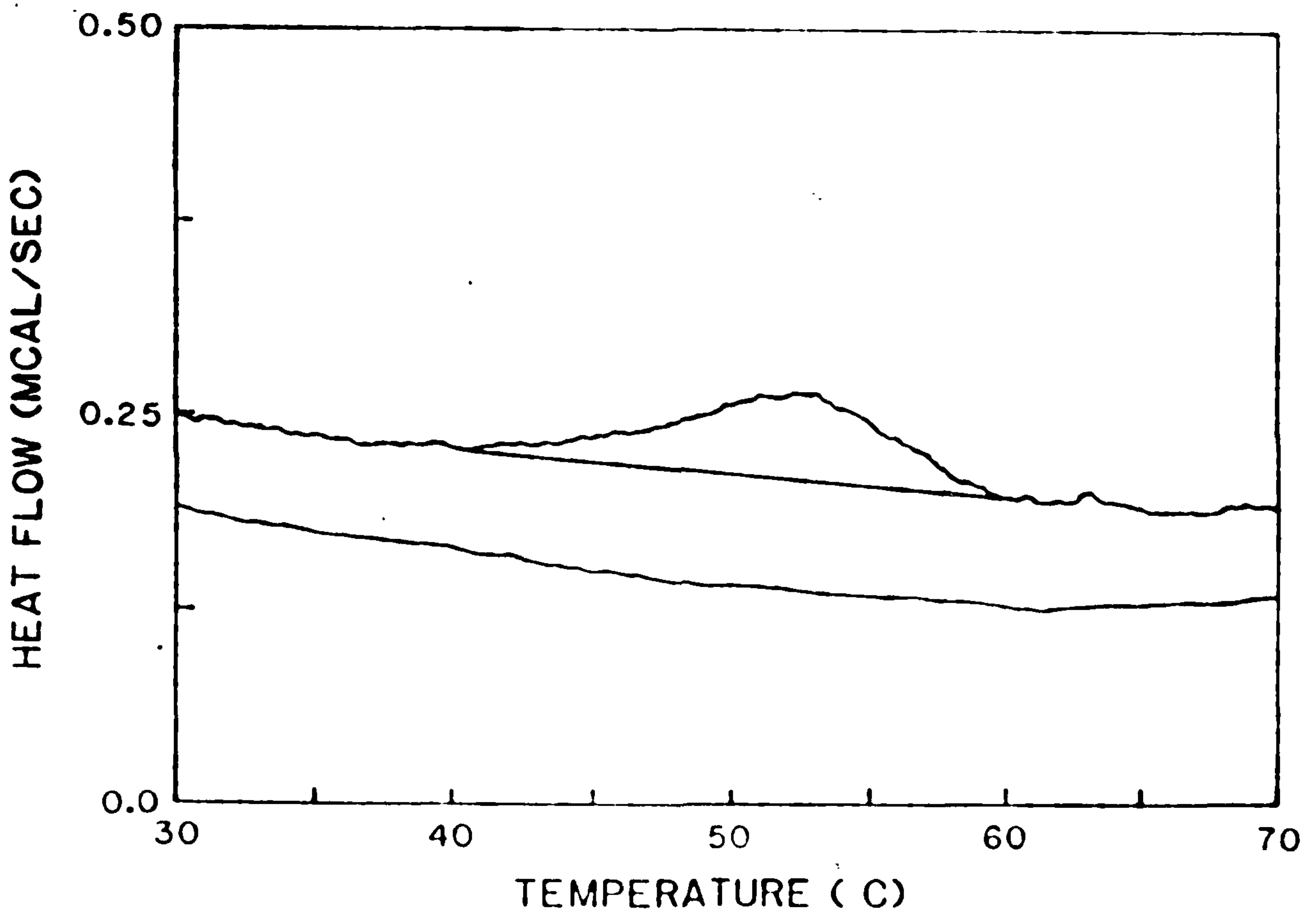

Figure 3. DSC thermal curves of fresh (lower) and 7-day (upper) bread crumb.

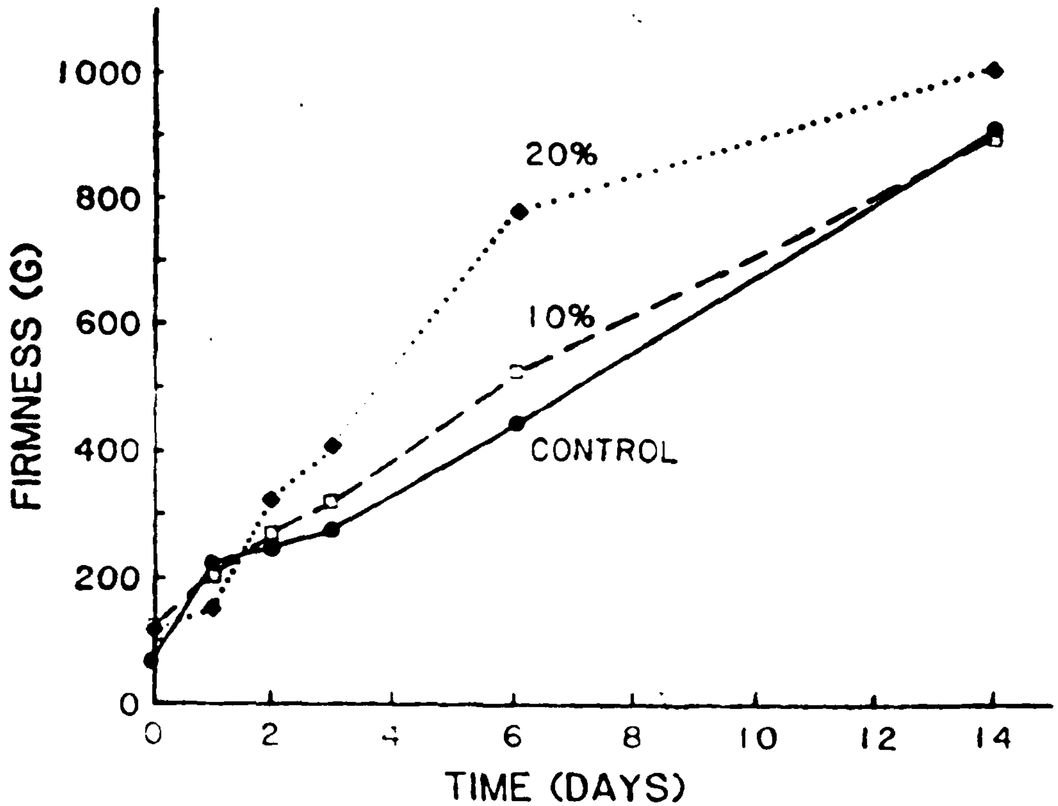

Figure 4. Effect of sorbitol and storage time on firmness of bread crumb.

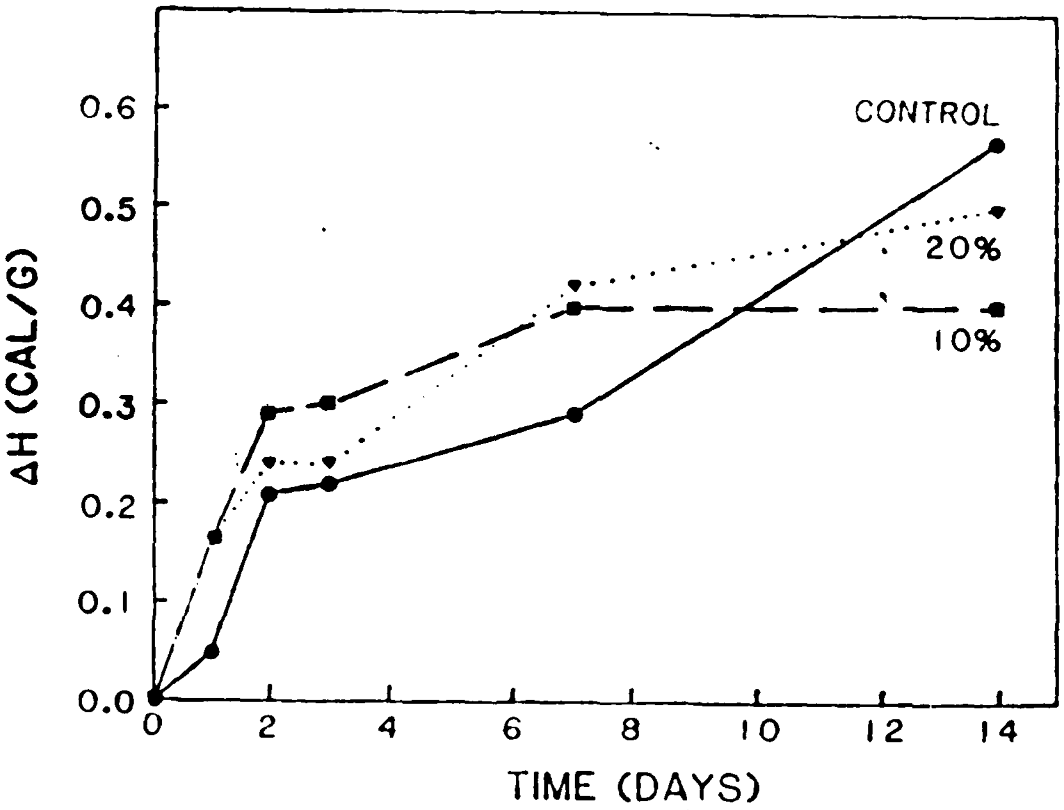

Figure 5. Effect of sorbitol and storage time on the area (enthalpy) of the DSC peak indicating starch crystallinity.

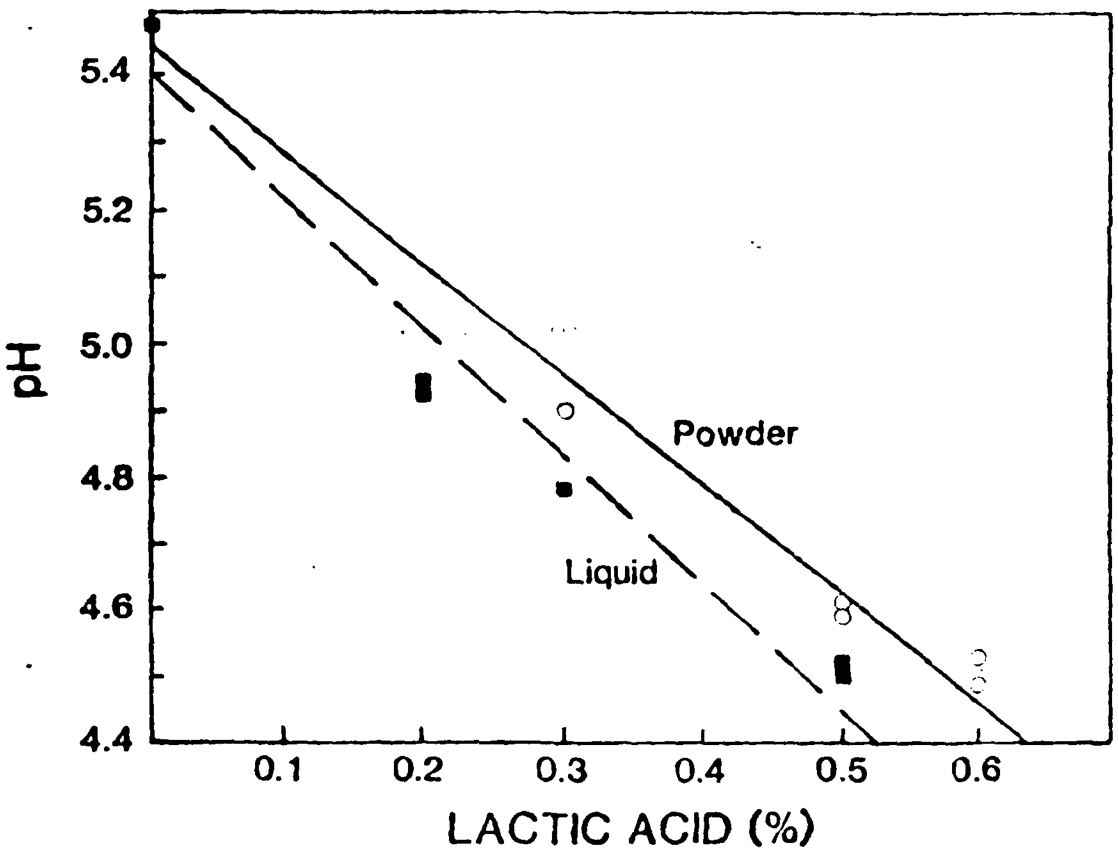

Figure 6. Effect of liquid and powder lactic acid concentrations on pH of humectant bread.

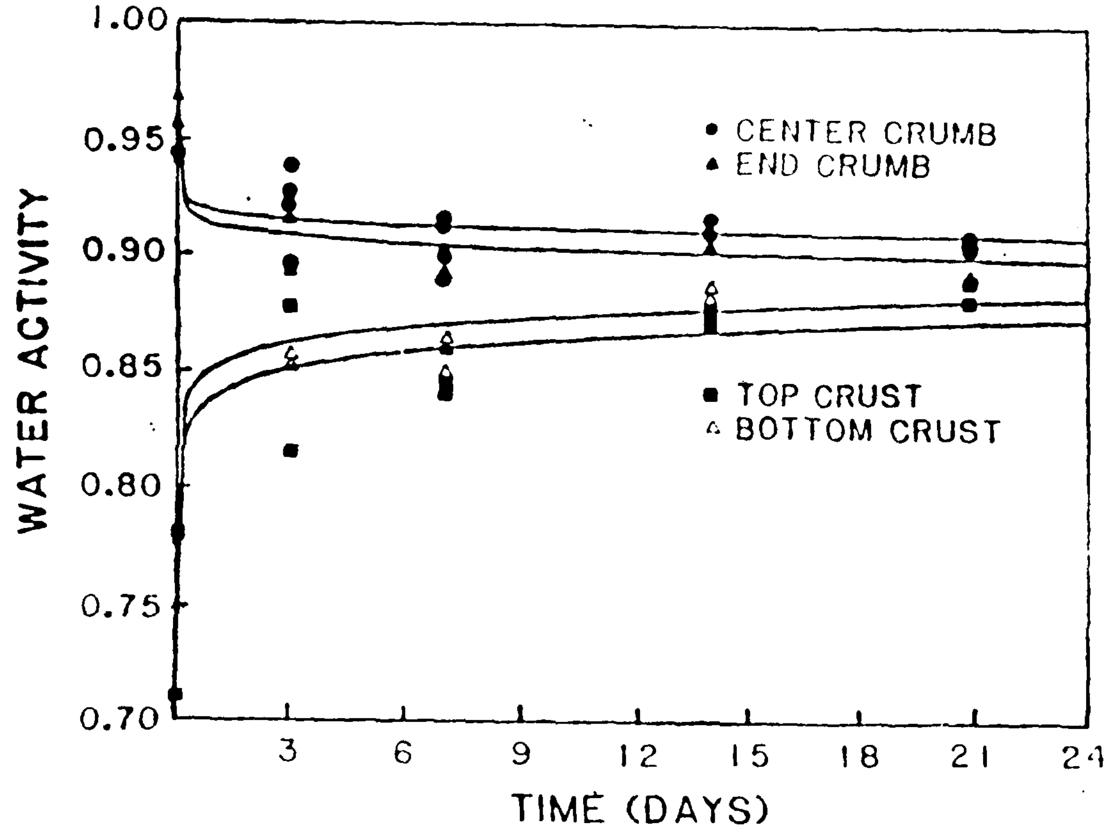

Figure 7. Effect of storage time on water activity of bread crumb at different locations of the loaf.

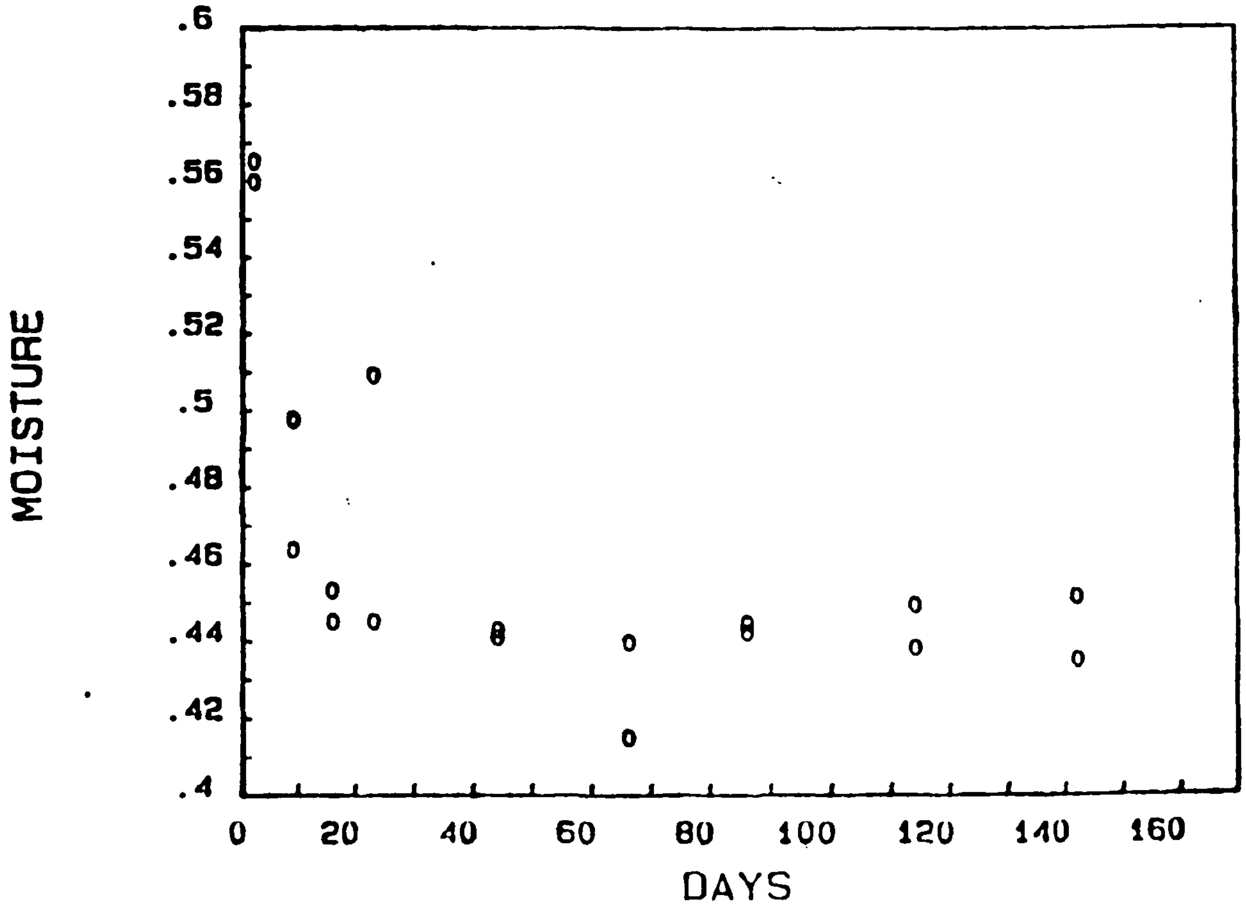

Figure 8. Moisture content of crumb in humectant bread (7-1-2) stored at 30°C.

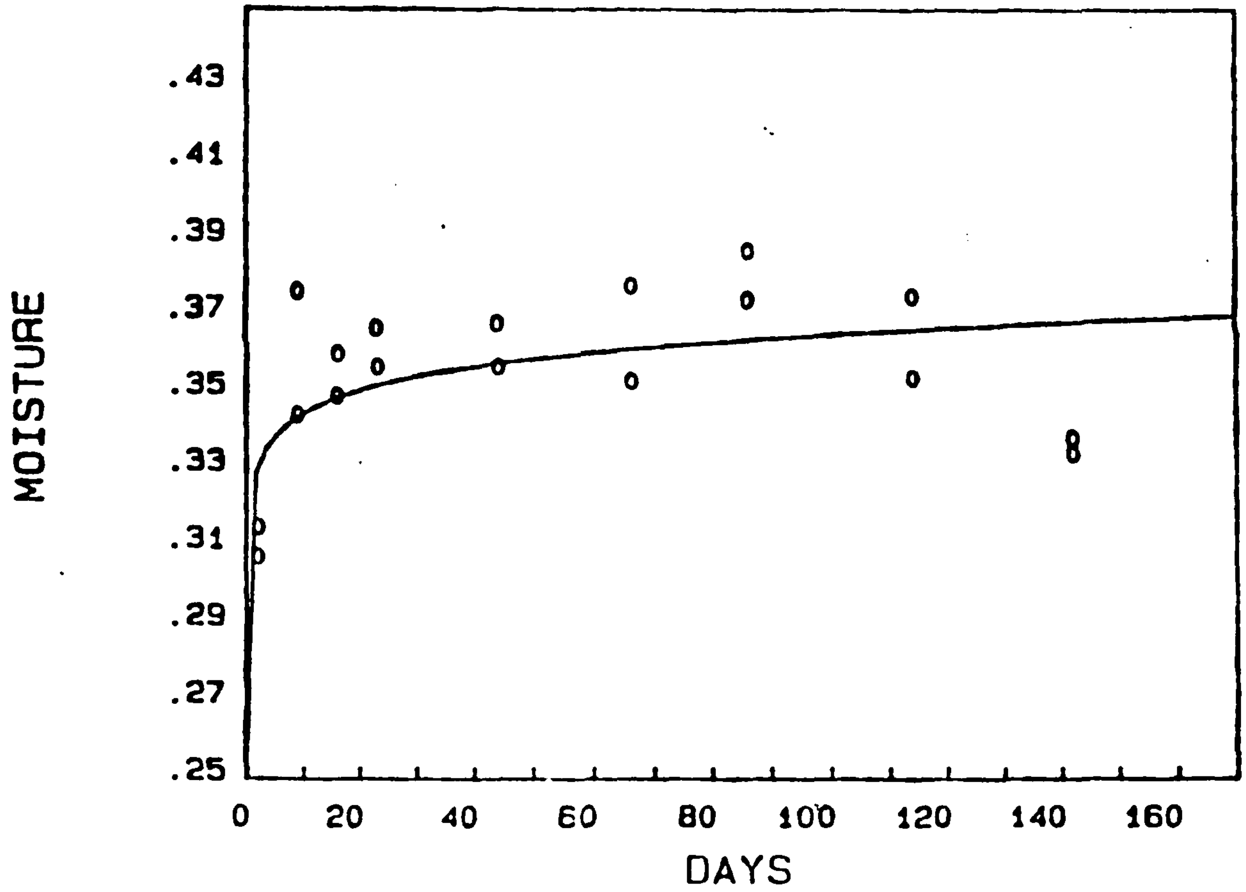

Figure 9. Moisture content of crust in humectant bread (7-1-2) stored at 30°C.

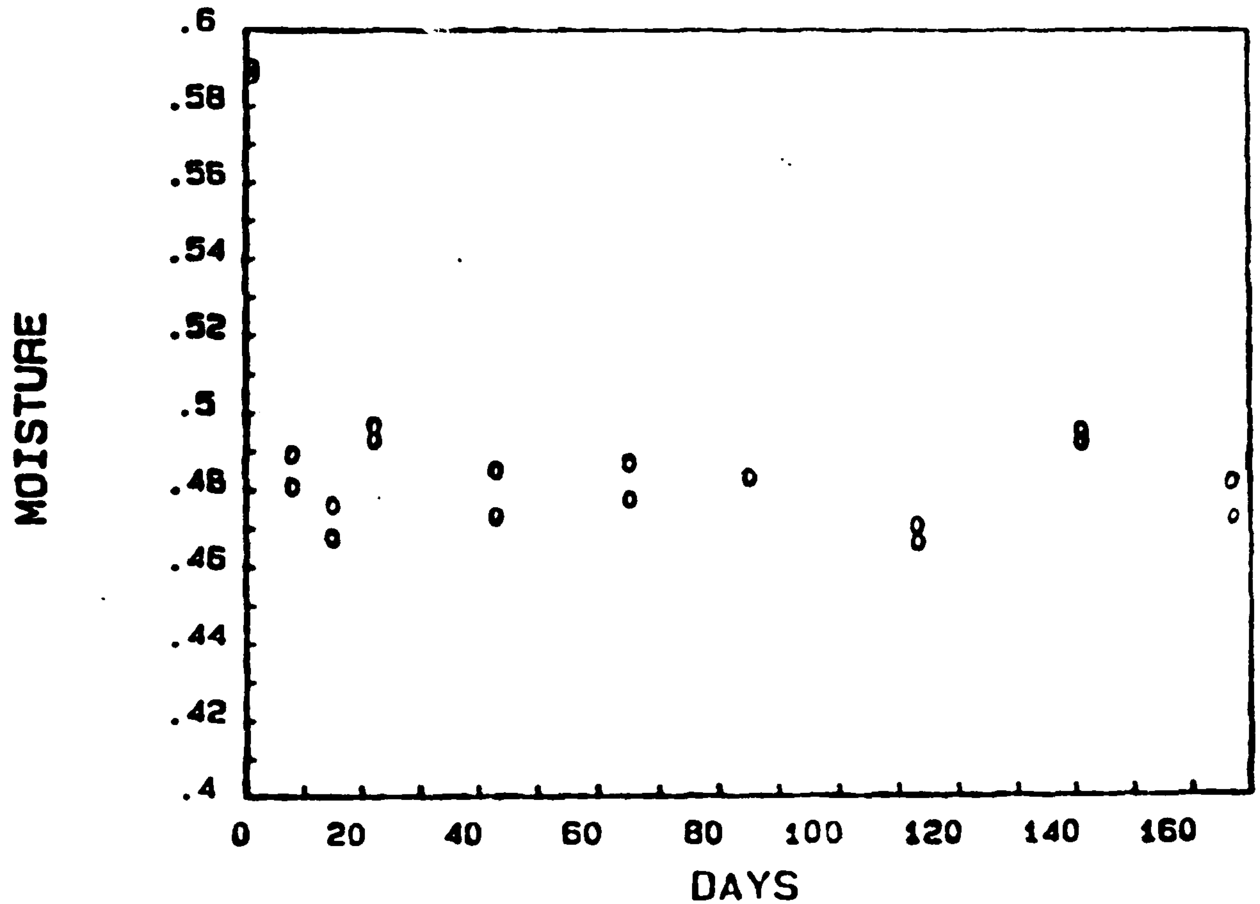

Figure 10. Moisture content of crumb in humectant bread (6-1.5-2) stored at 30^{0}C.

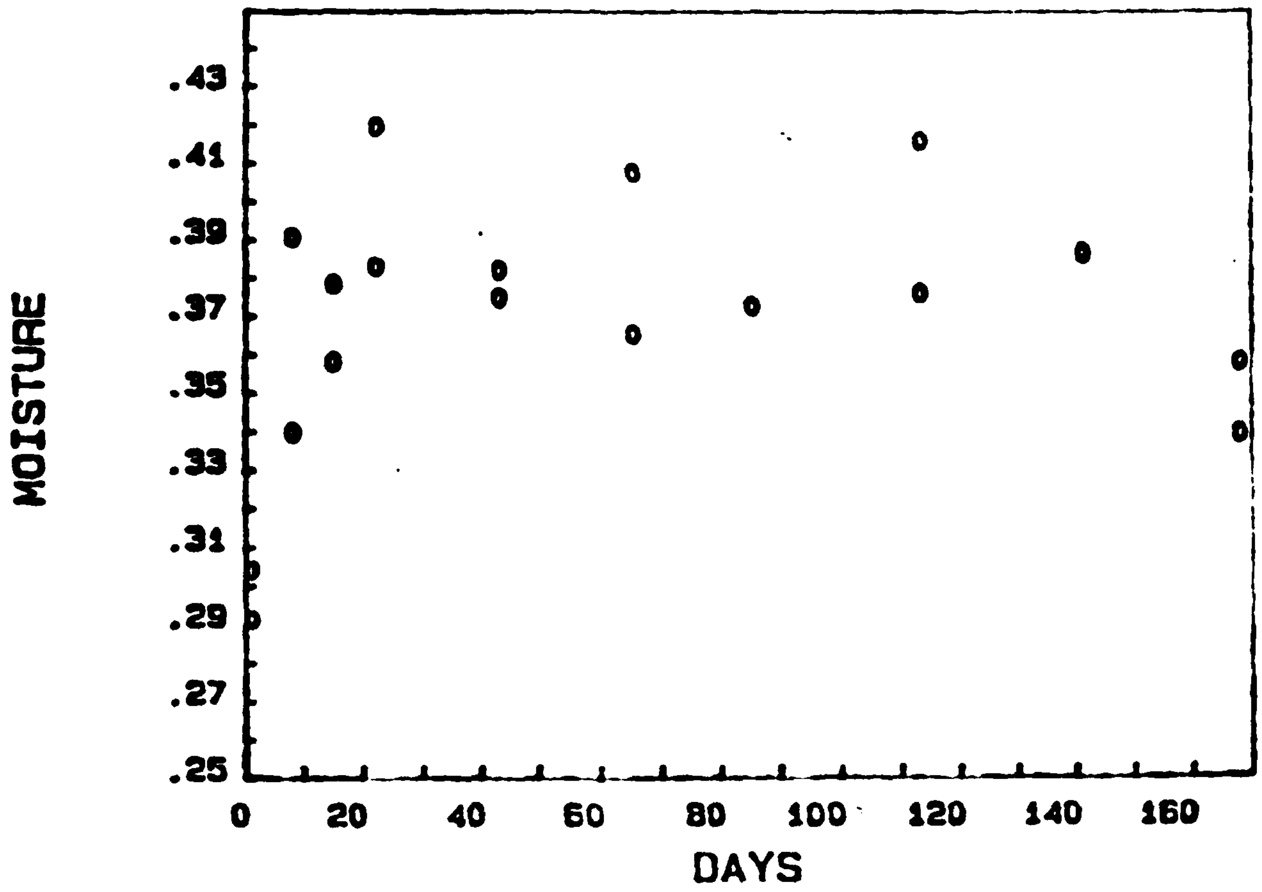

Figure 11. Moisture content of crust in humectant bread (6-1.5-2) stored at 30°C.

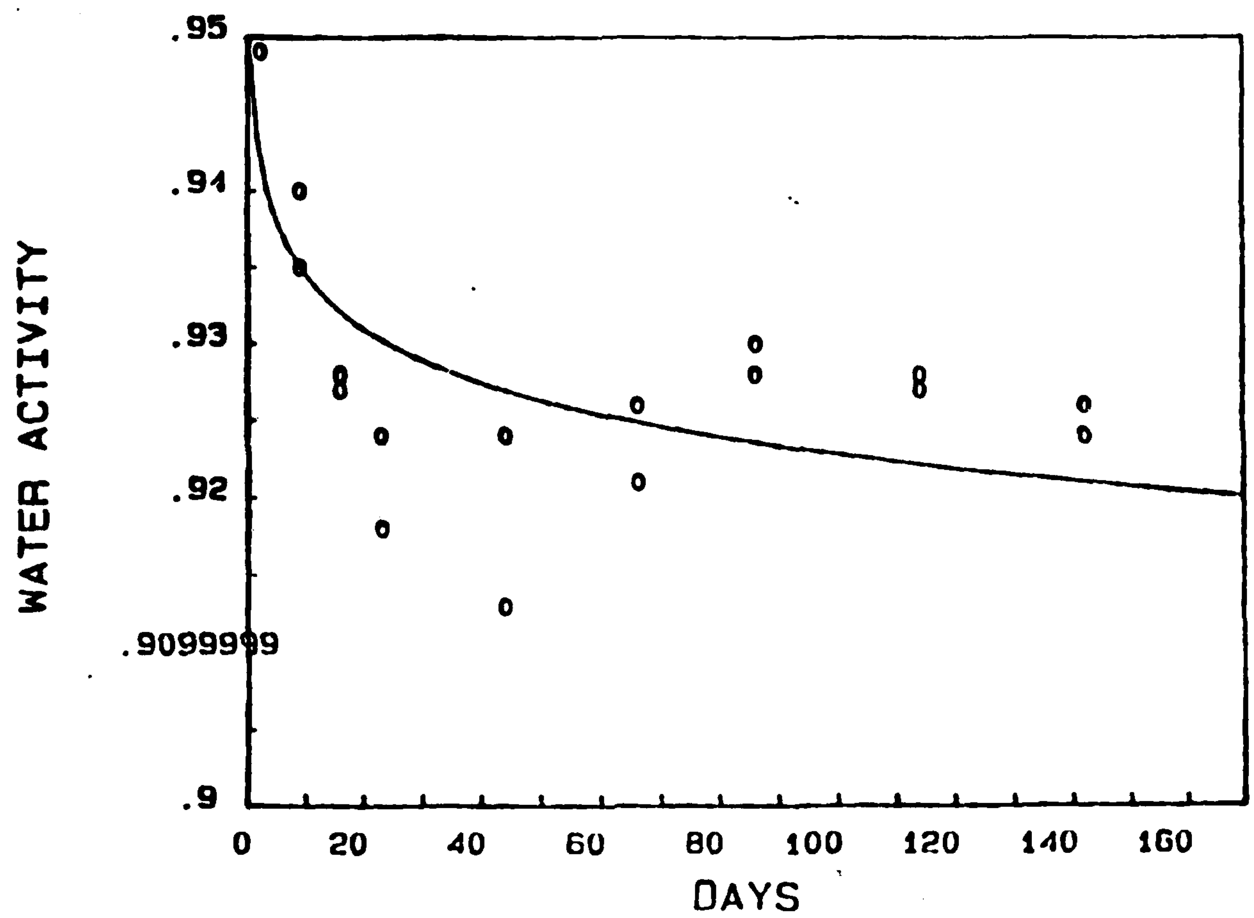

Figure 12. Water activity of crumb in humectant bread (7-1-2) stored at 30^{o}C.

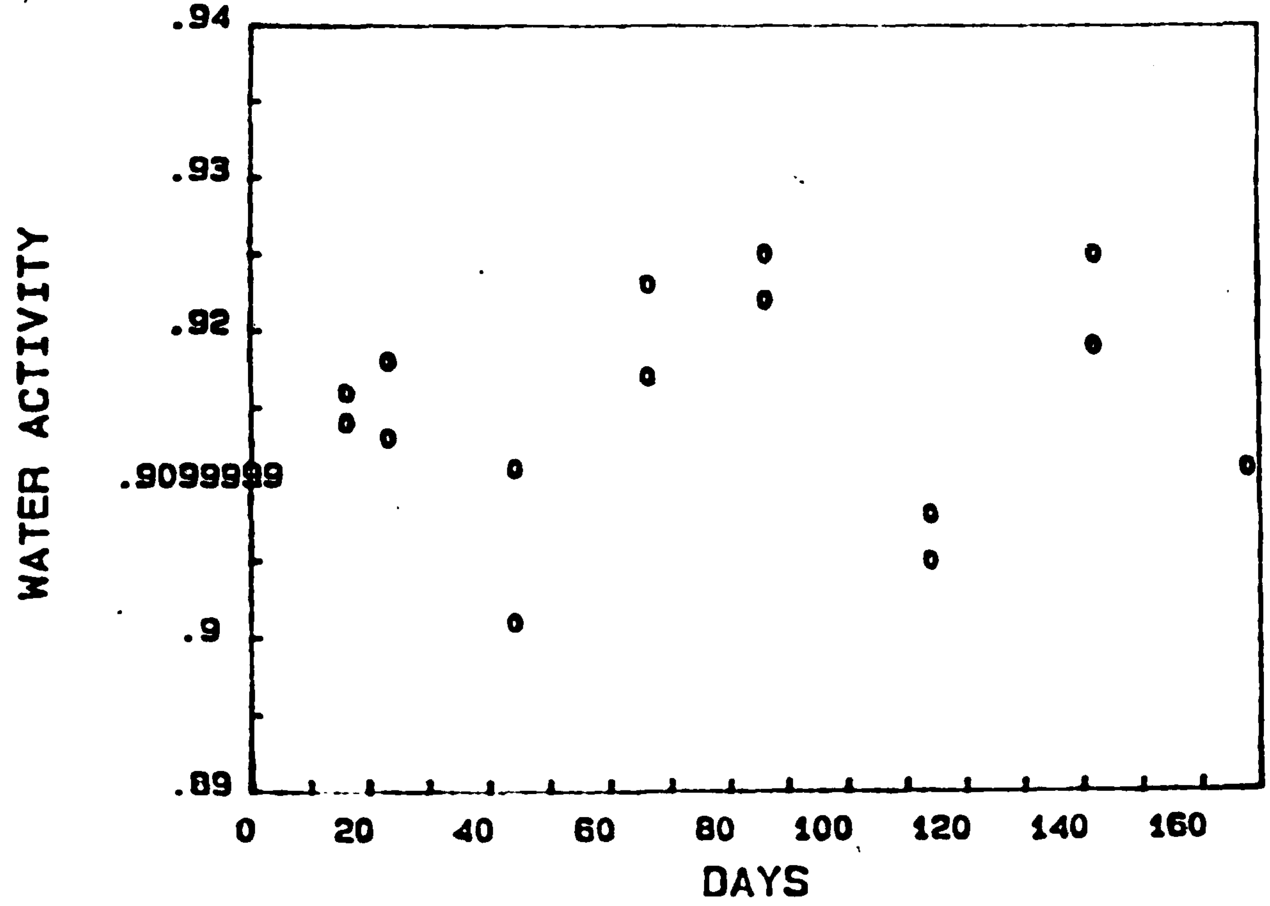

Figure 13. Water activity of crust in humectant bread (7-1-2) stored at 30°C.

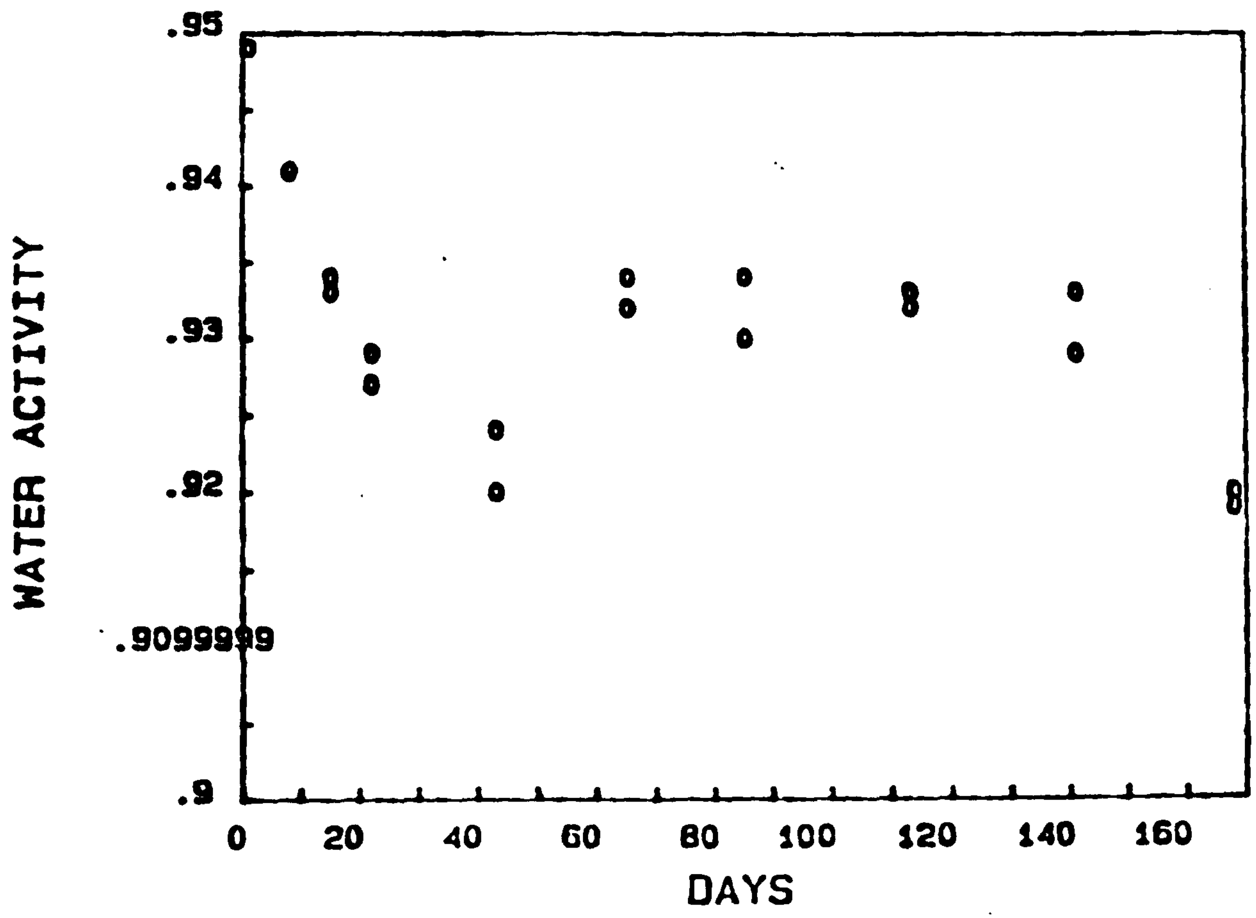

Figure 14. Water activity of crumb in humectant bread (6-1.5-2) stored at 30°C.

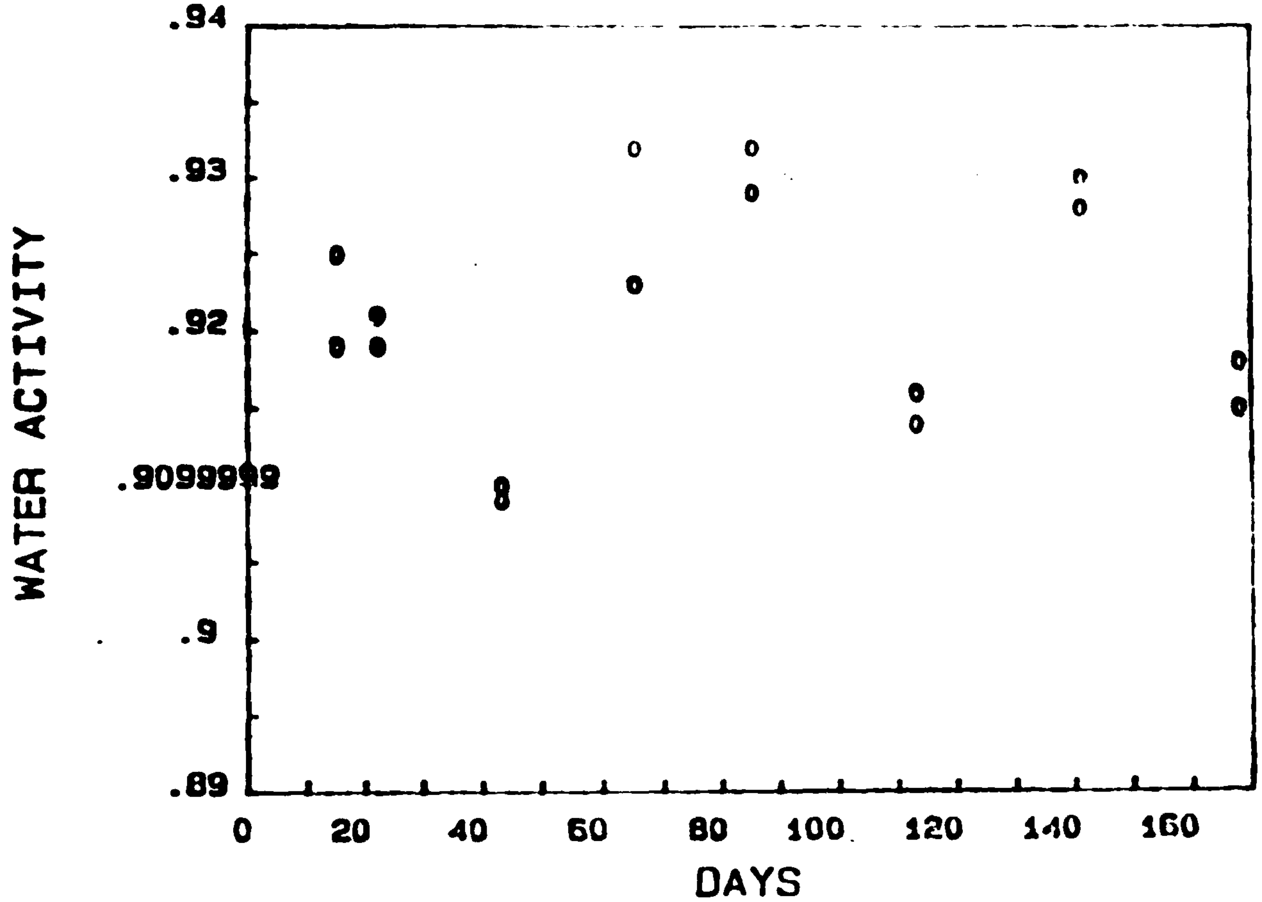

Figure 15. Water activity of crust in humectant bread stored at 30°C.

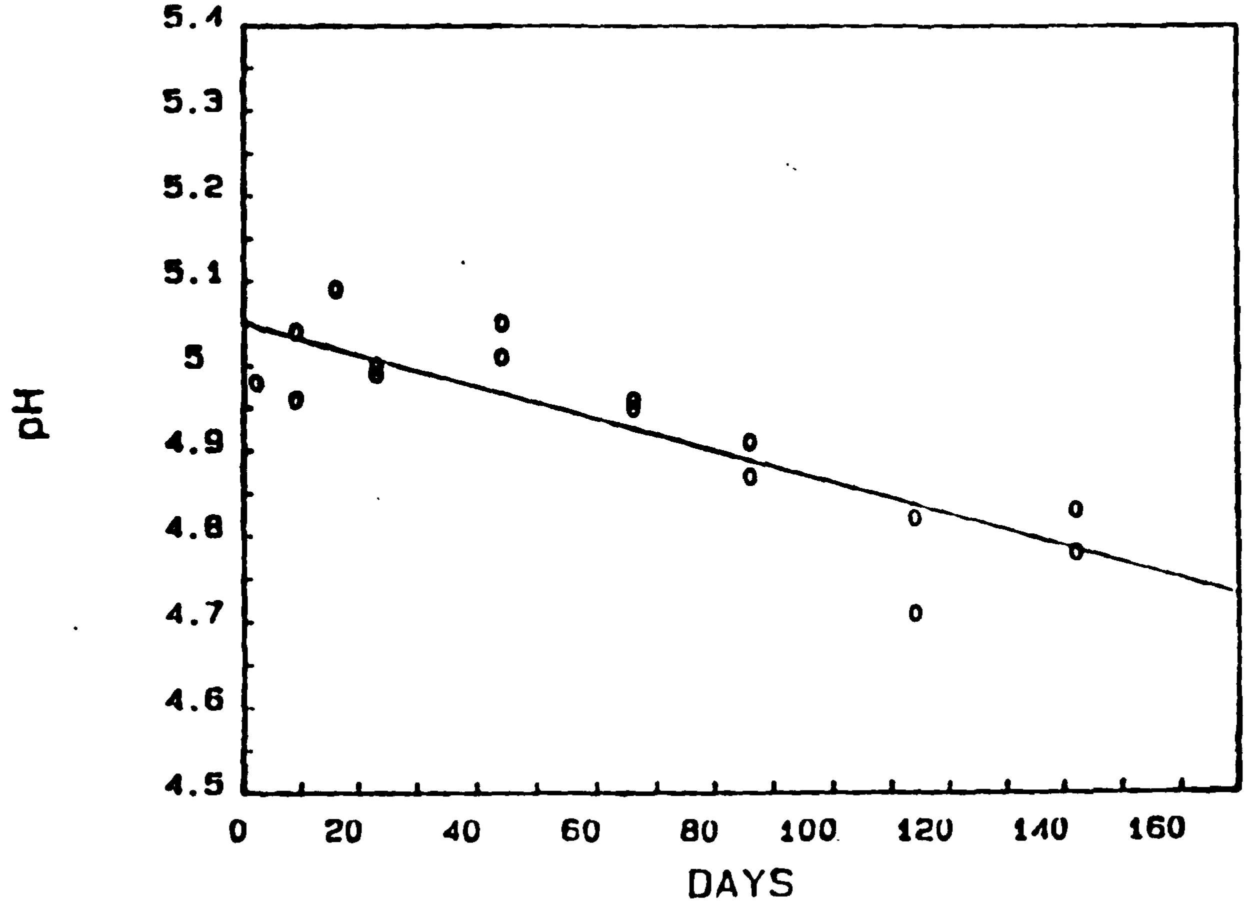

Figure 16. Effect of storage time at 30^{0}C on pH of humectant bread (7-1-2).

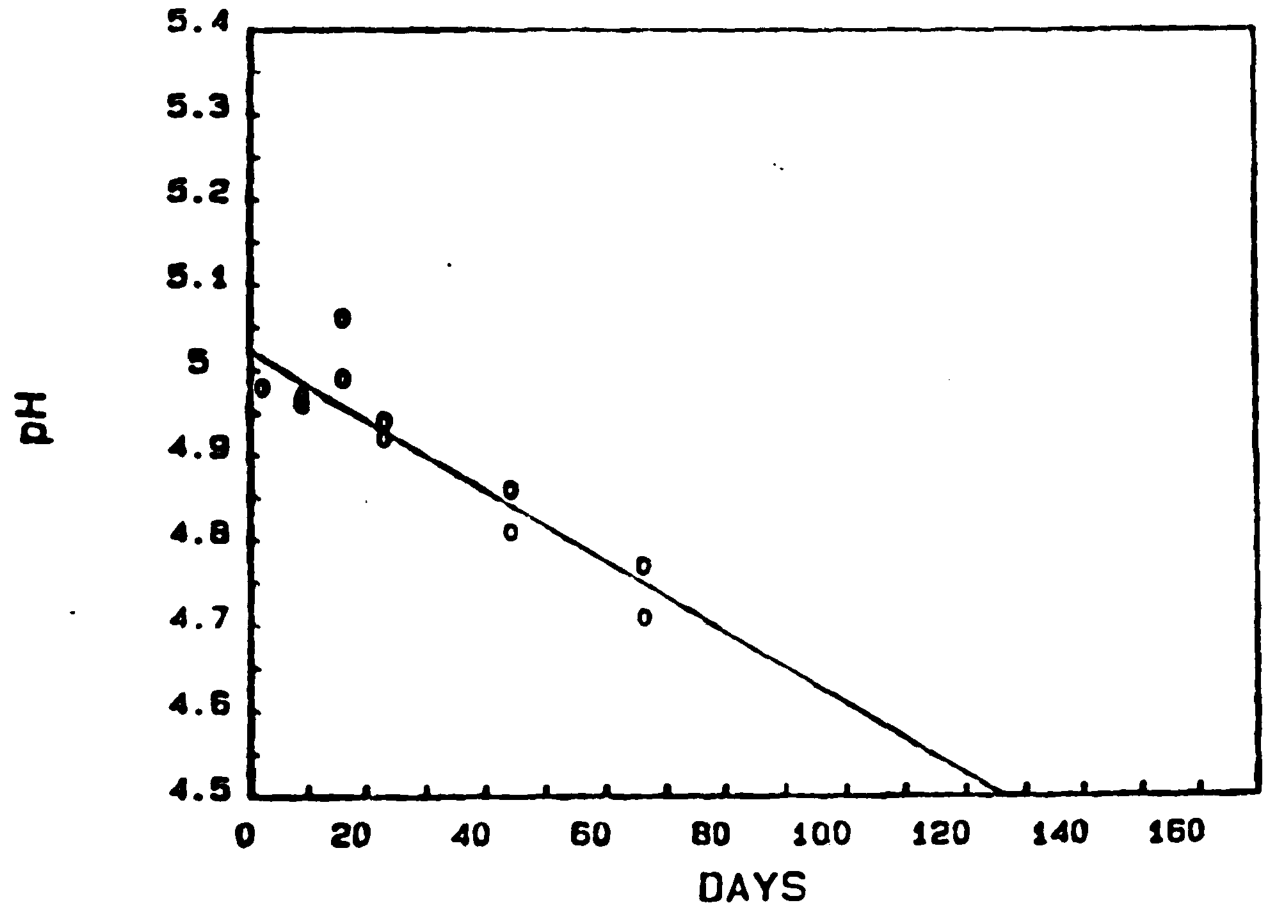

Figure 17. Effect of storage time at 45°C on pH of humectant bread (7-1-2).

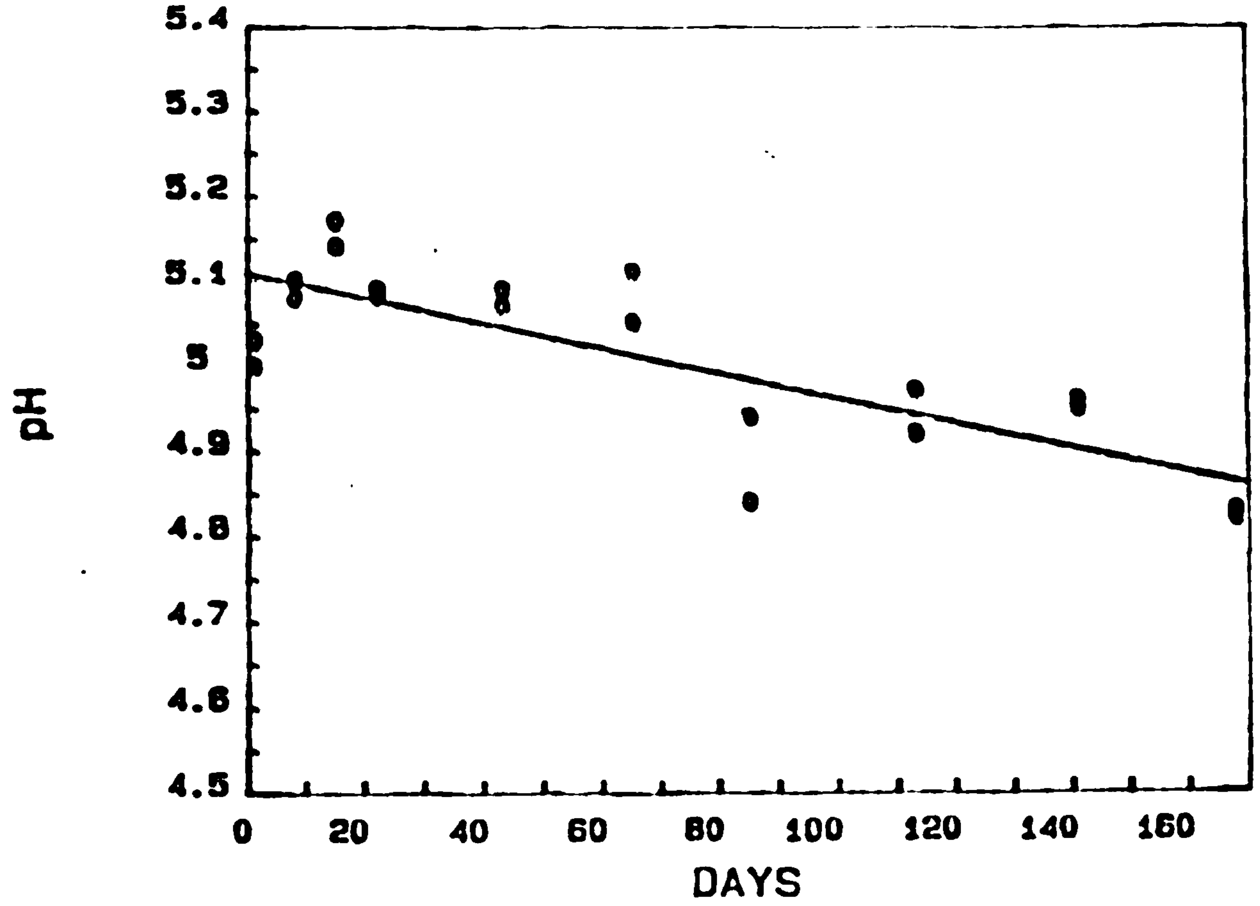

Figure 18. Effect of storage time at $30^{\circ}C$ on pH of humectant bread (6-1.5-2).

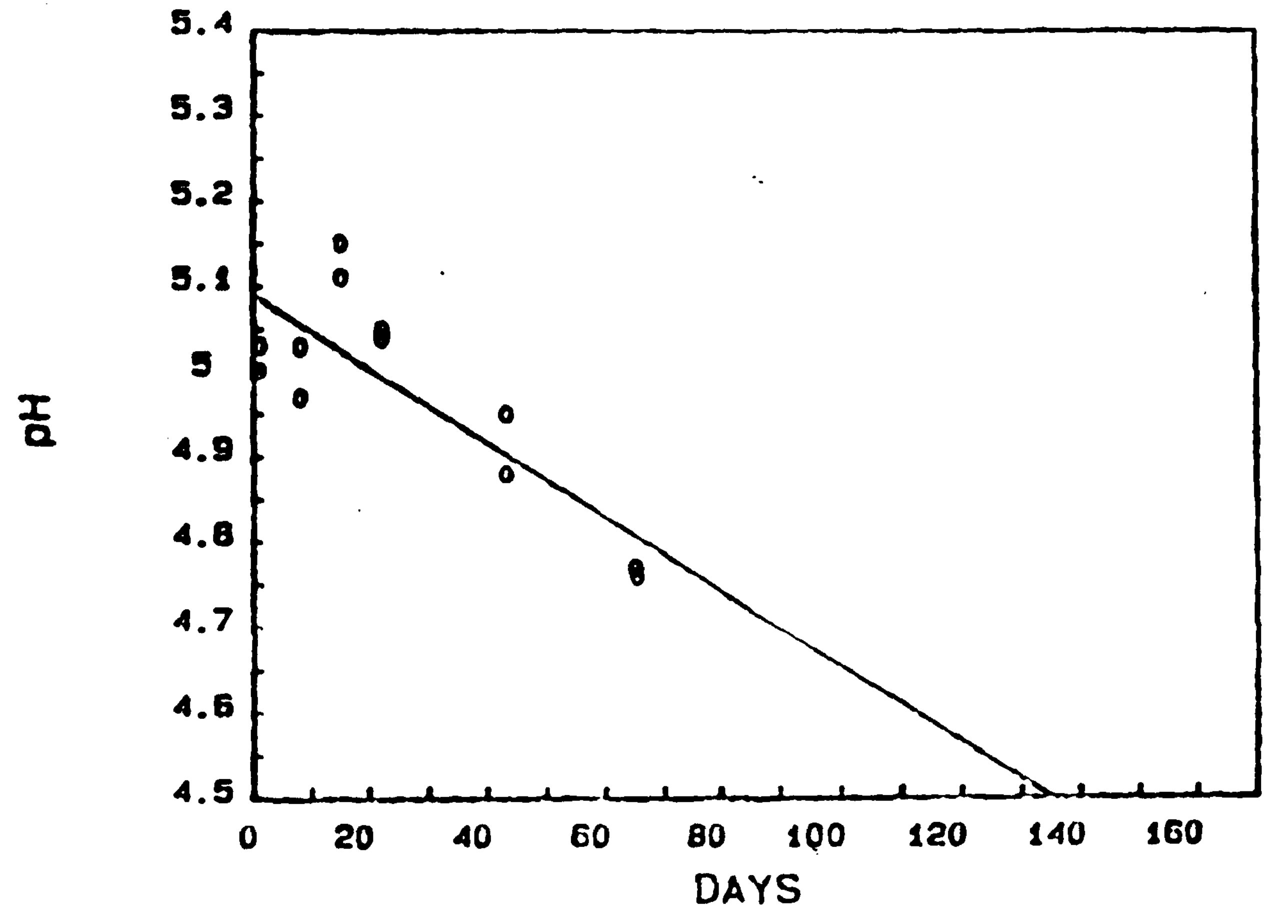

Figure 19. Effect of storage time at $45^{\circ}C$ on pH of humectant bread (6-1.5-2).

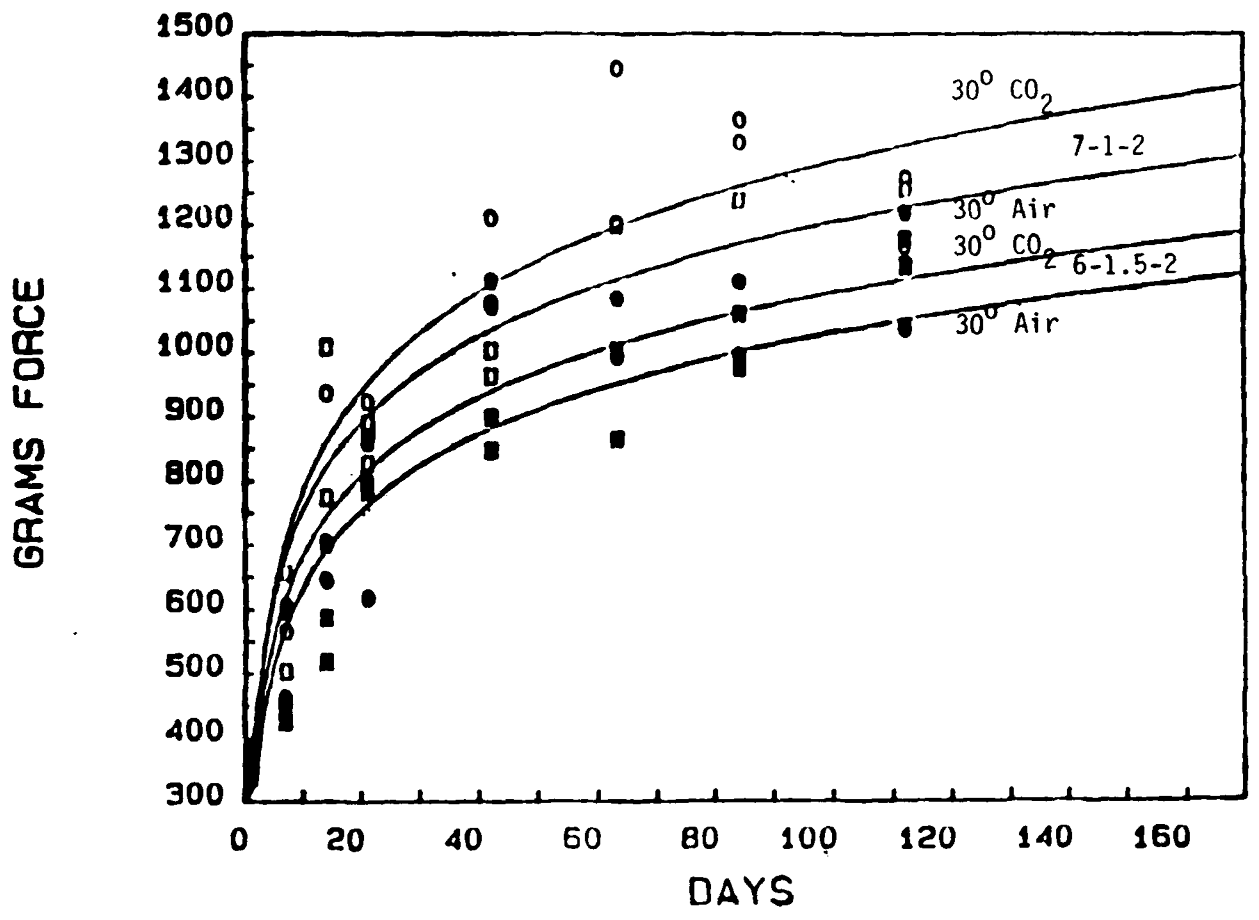

Figure 20. Effect of storage time at 30°C on firmness of humectant breads stored in air and CO_2.

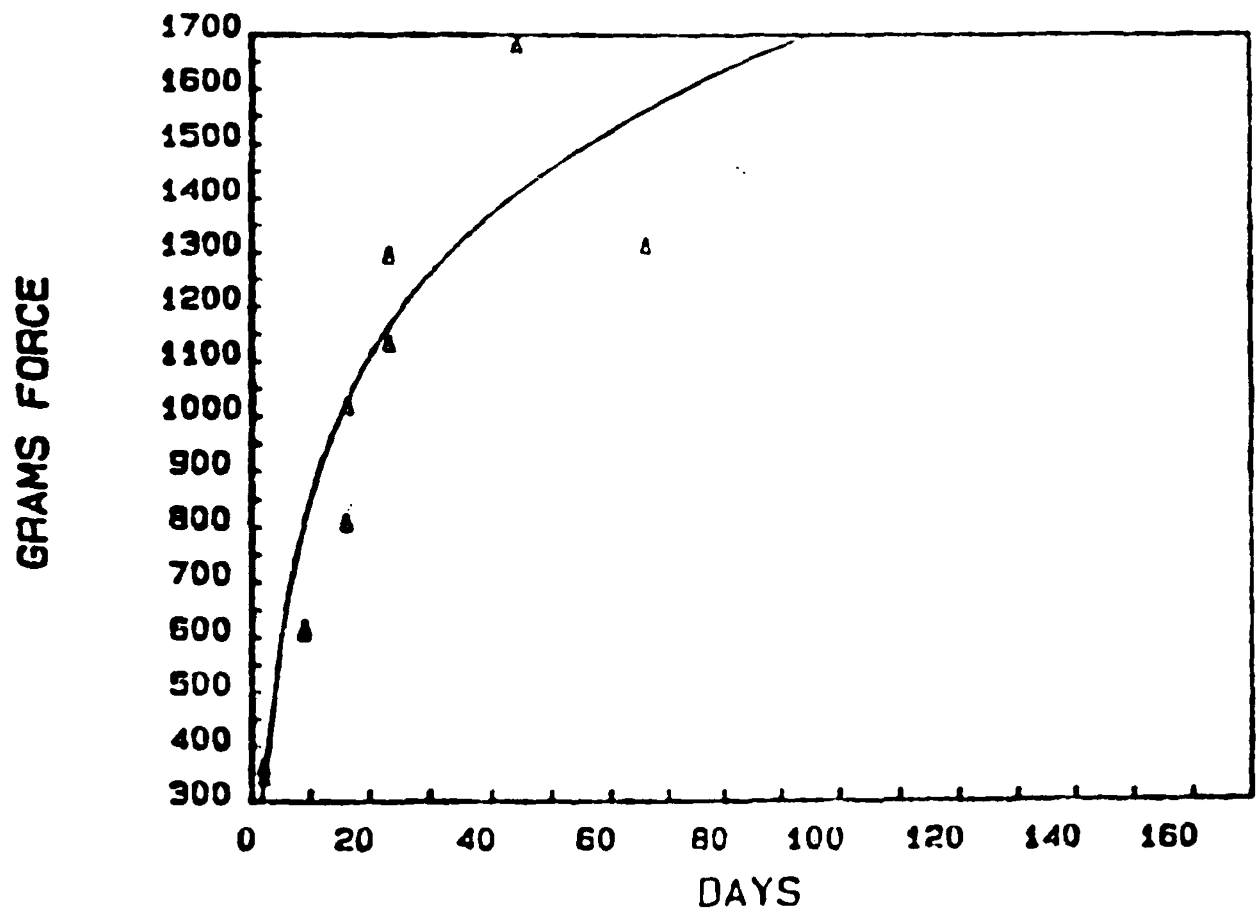

Figure 21. Effect of storage time at 45°C on firmness of humectant bread (7-1-2) stored in CO_2.

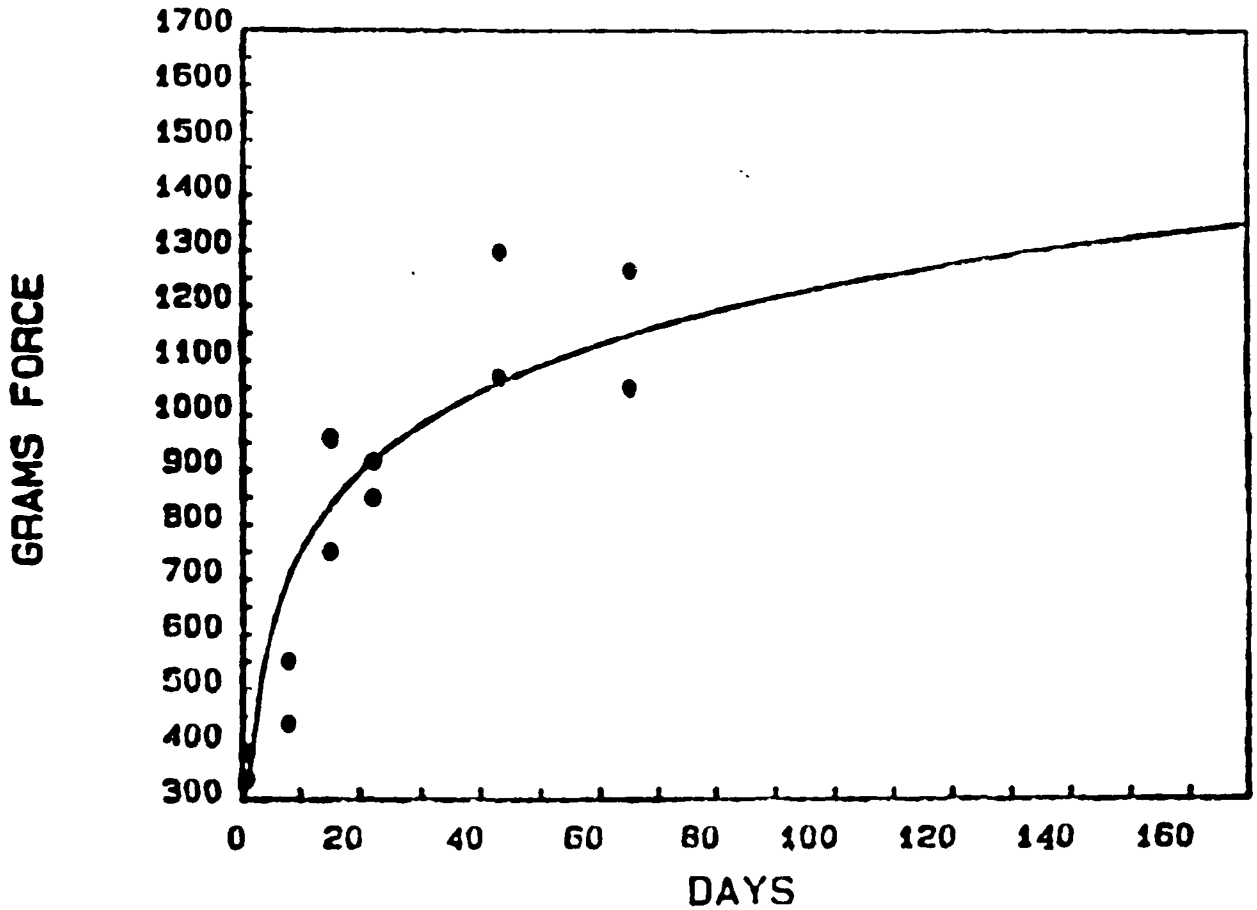

Figure 22. Effect of storage time at 45°C on firmness of humectant bread (6-1.5-2) stored in CO_2.

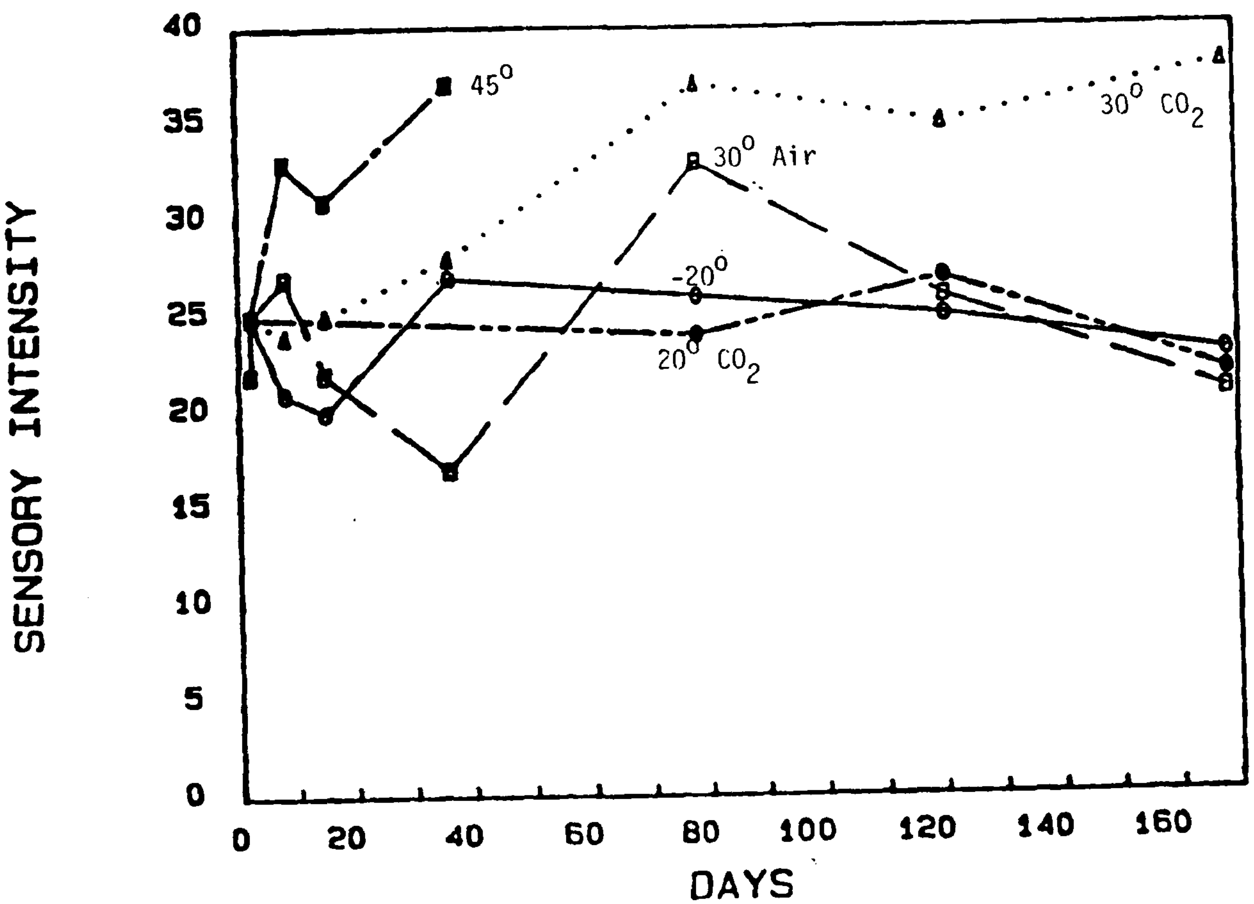

Figure 23. Effect of storage conditions on crust browning of humectant bread (7-1-2).

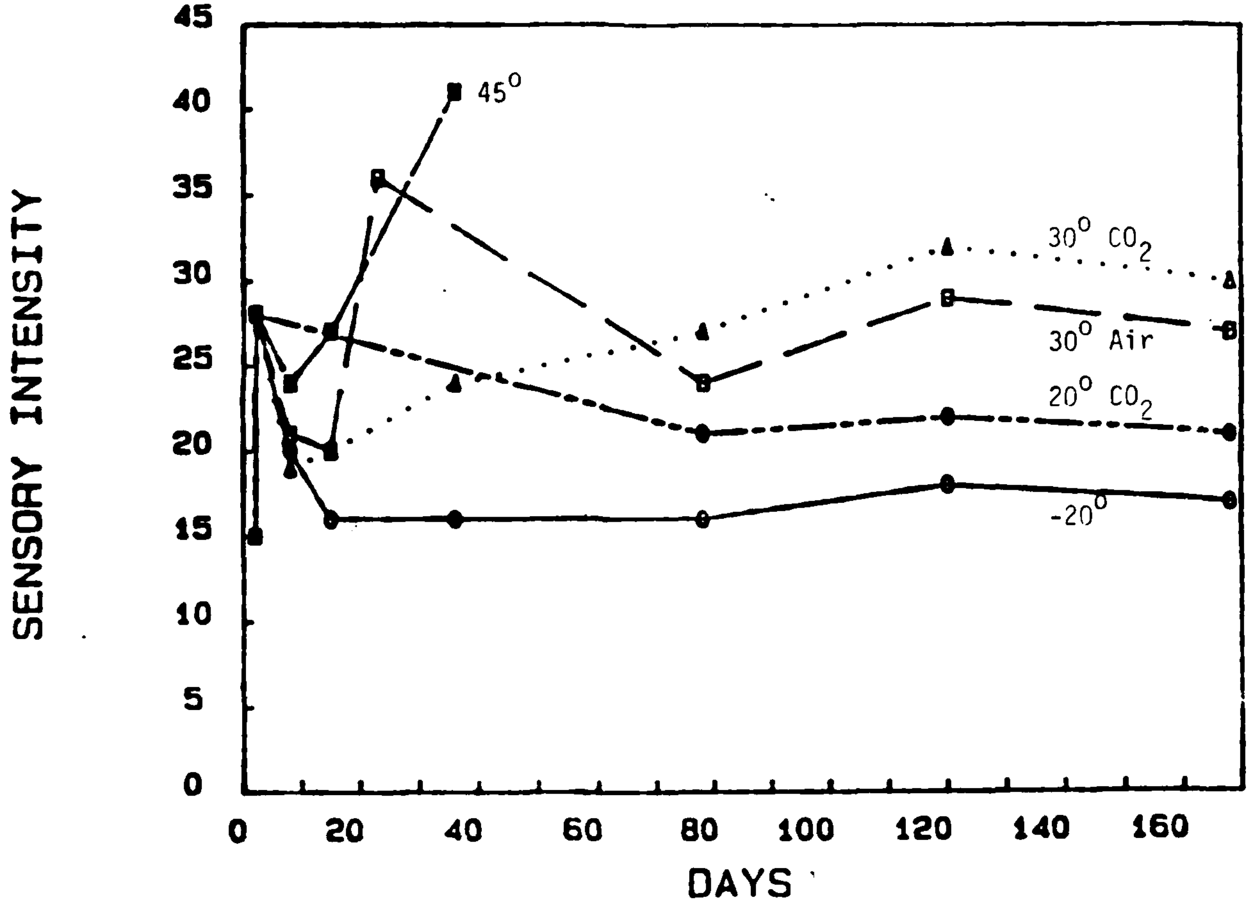

Figure 24. Effect of storage conditions on crumb color of humectant bread (7-1-2).

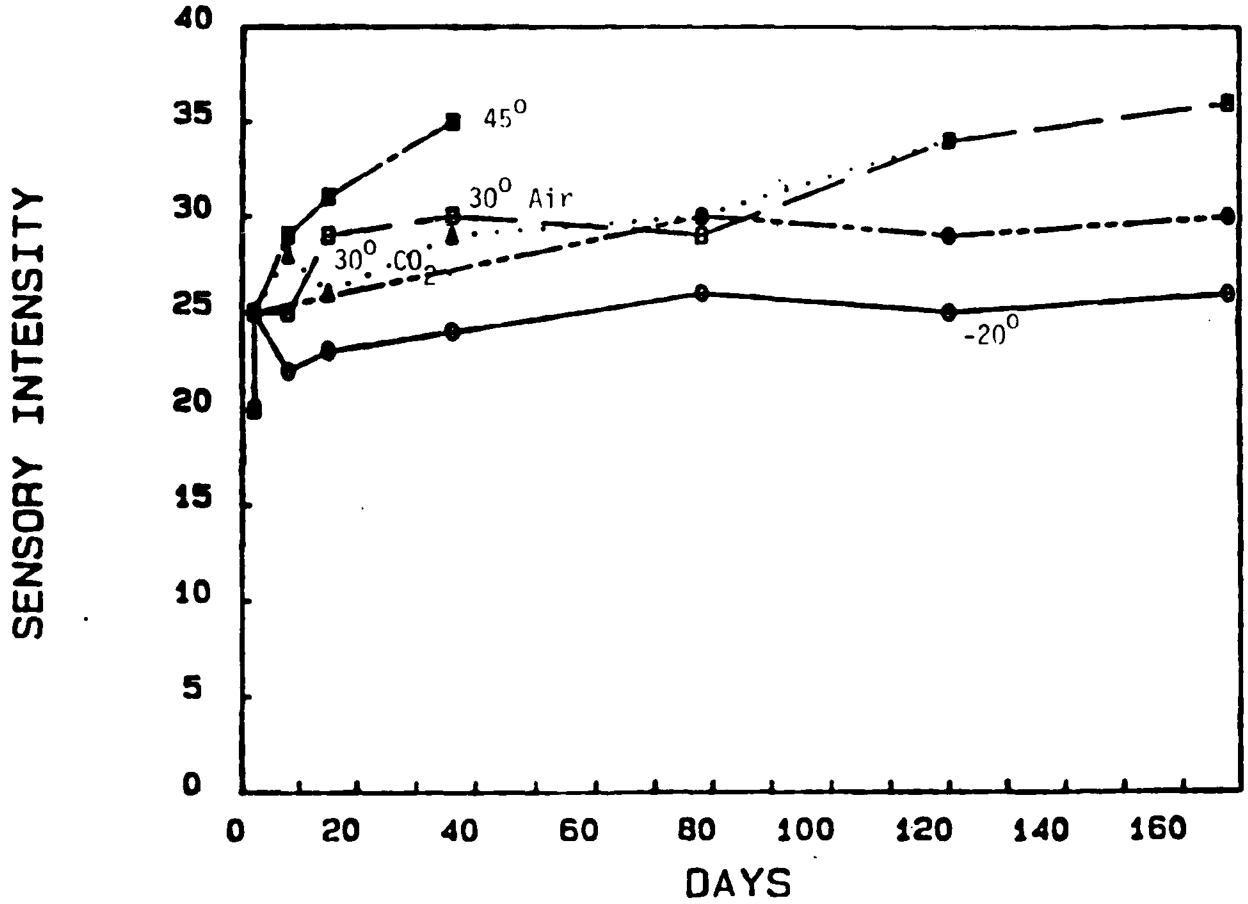

Figure 25. Effect of storage conditions on overall aroma intensity of humectant bread (7-1-2).

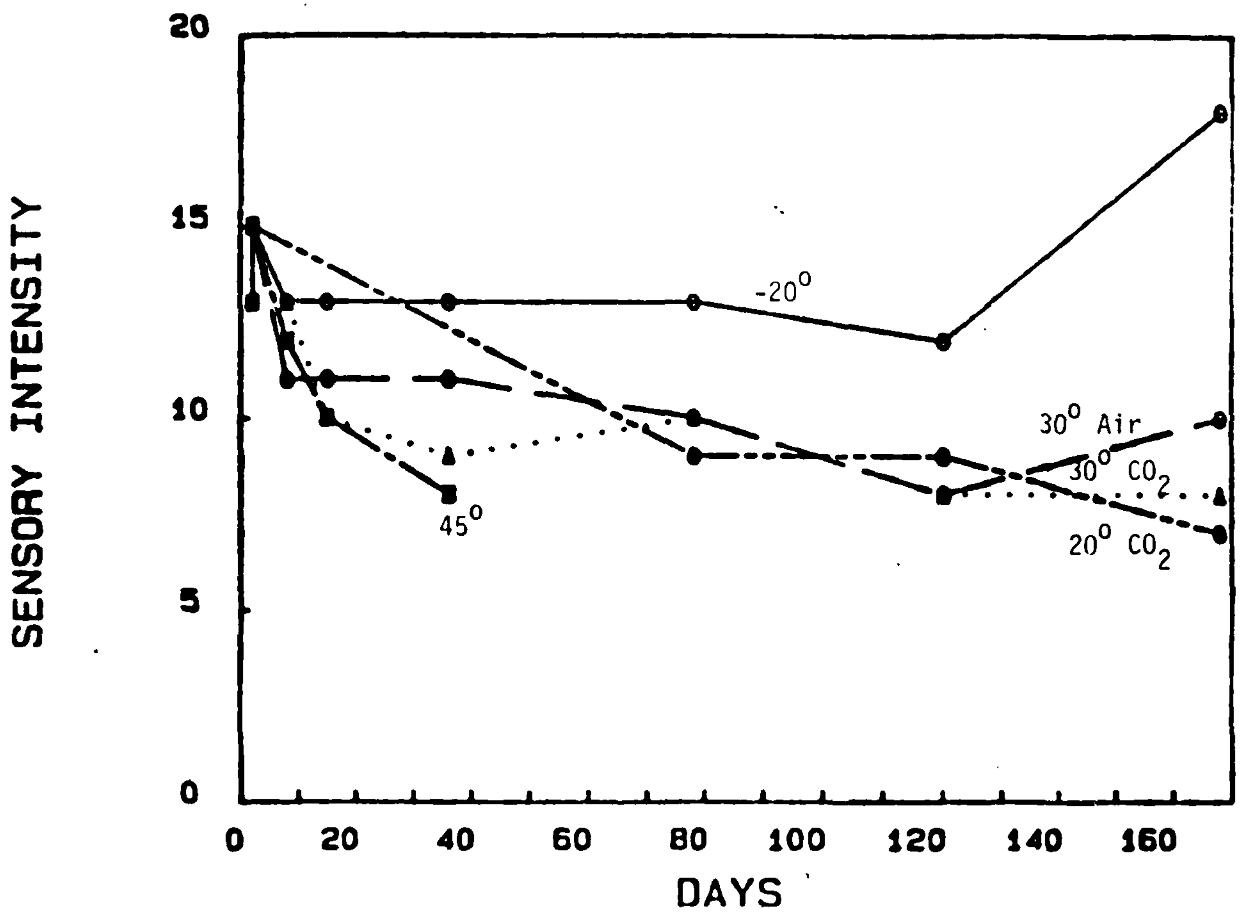

Figure 26. Effect of storage conditions on yeast aroma of humectant bread (7-1-2).

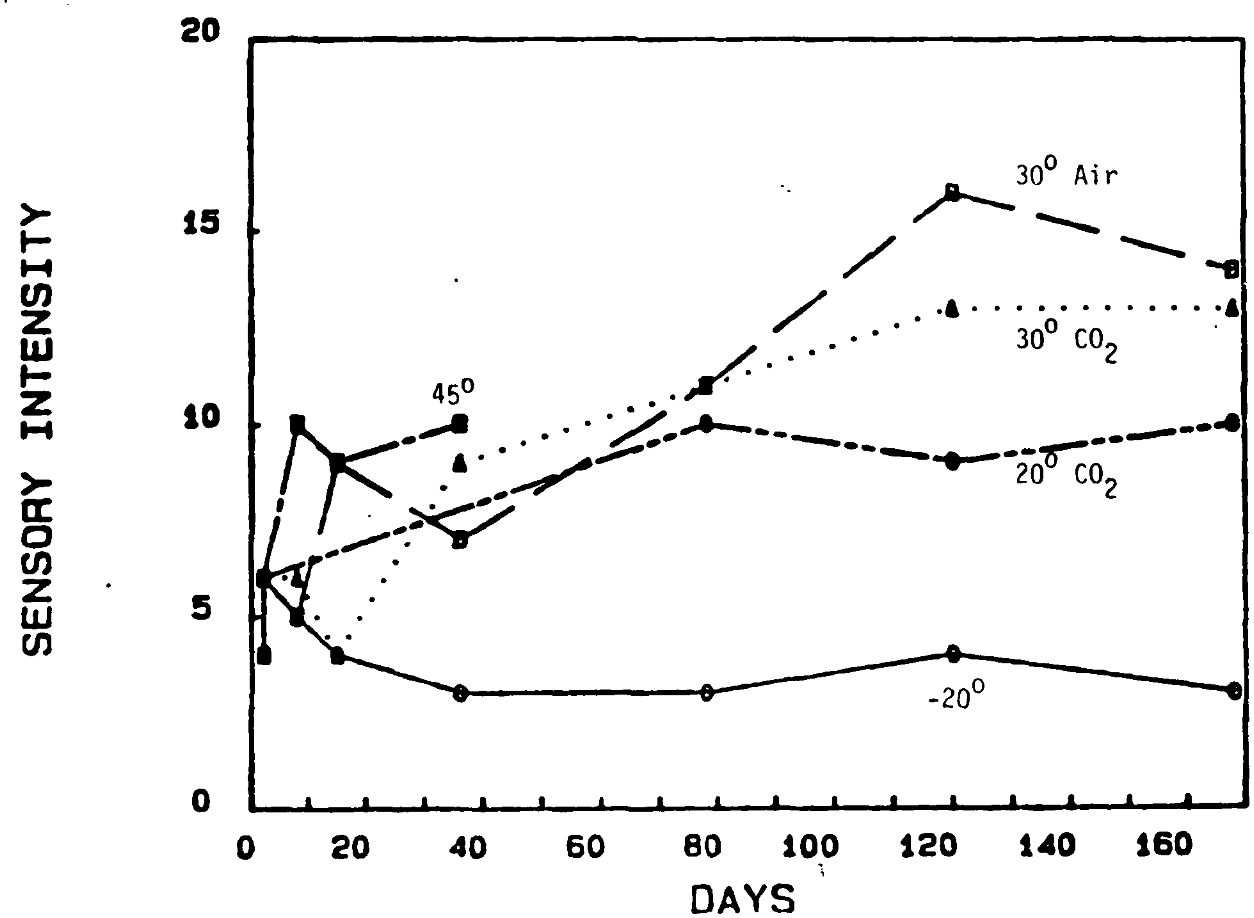

Figure 27. Effect of storage conditions on acrid aroma of humectant bread (7-1-2).

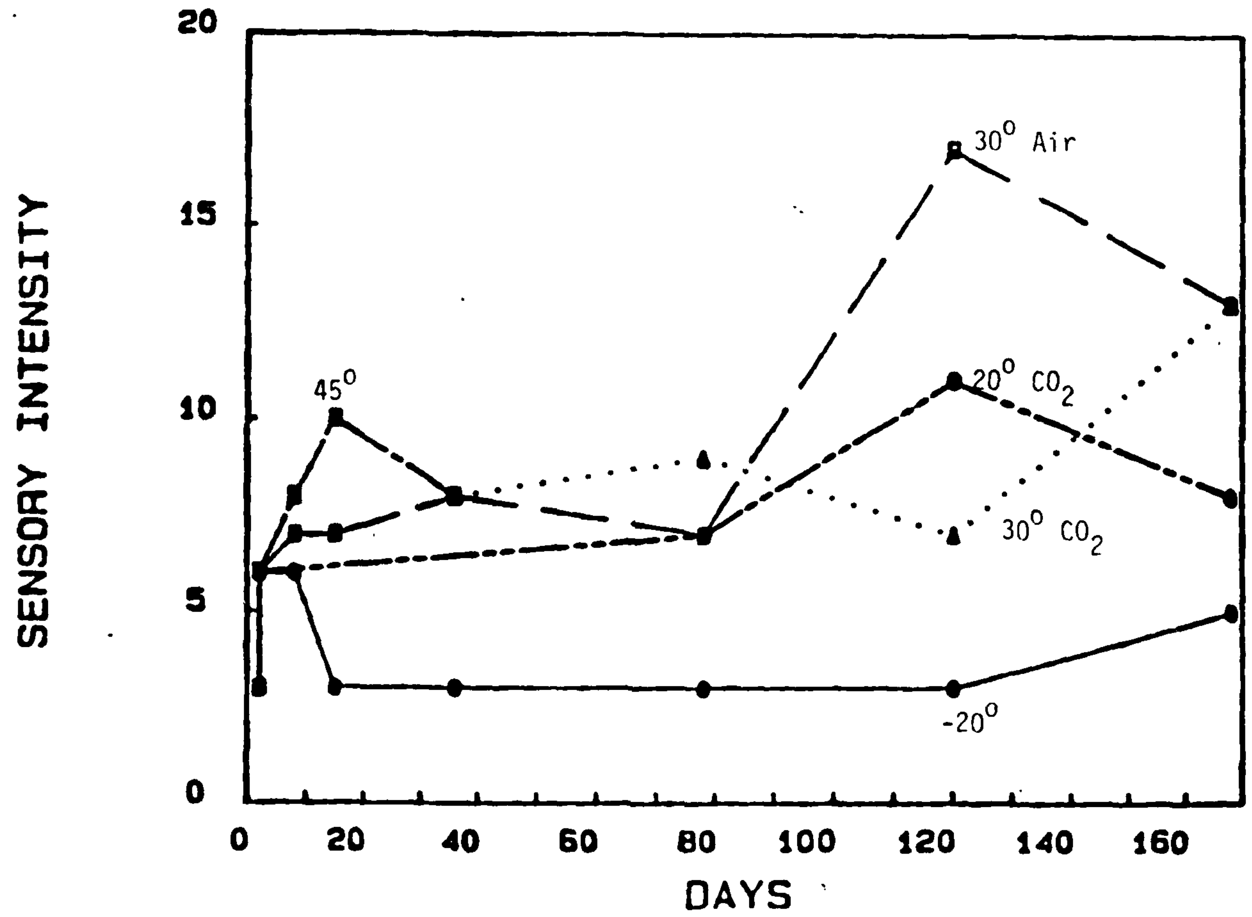

Figure 28. Effect of storage conditions on sour aroma of humectant bread (7-1-2).

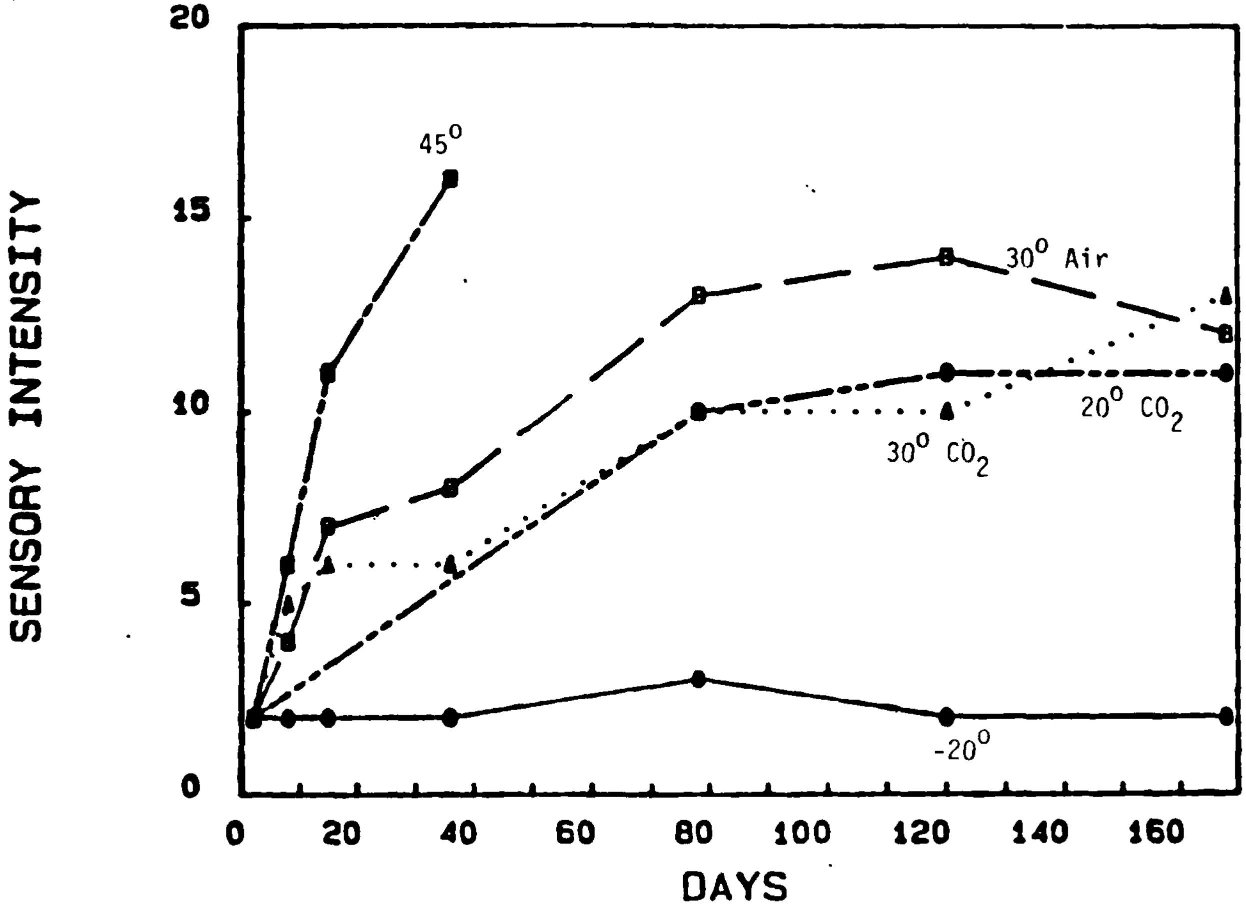

Figure 29. Effect of storage conditions on rancid aroma of humectant bread (7-1-2).

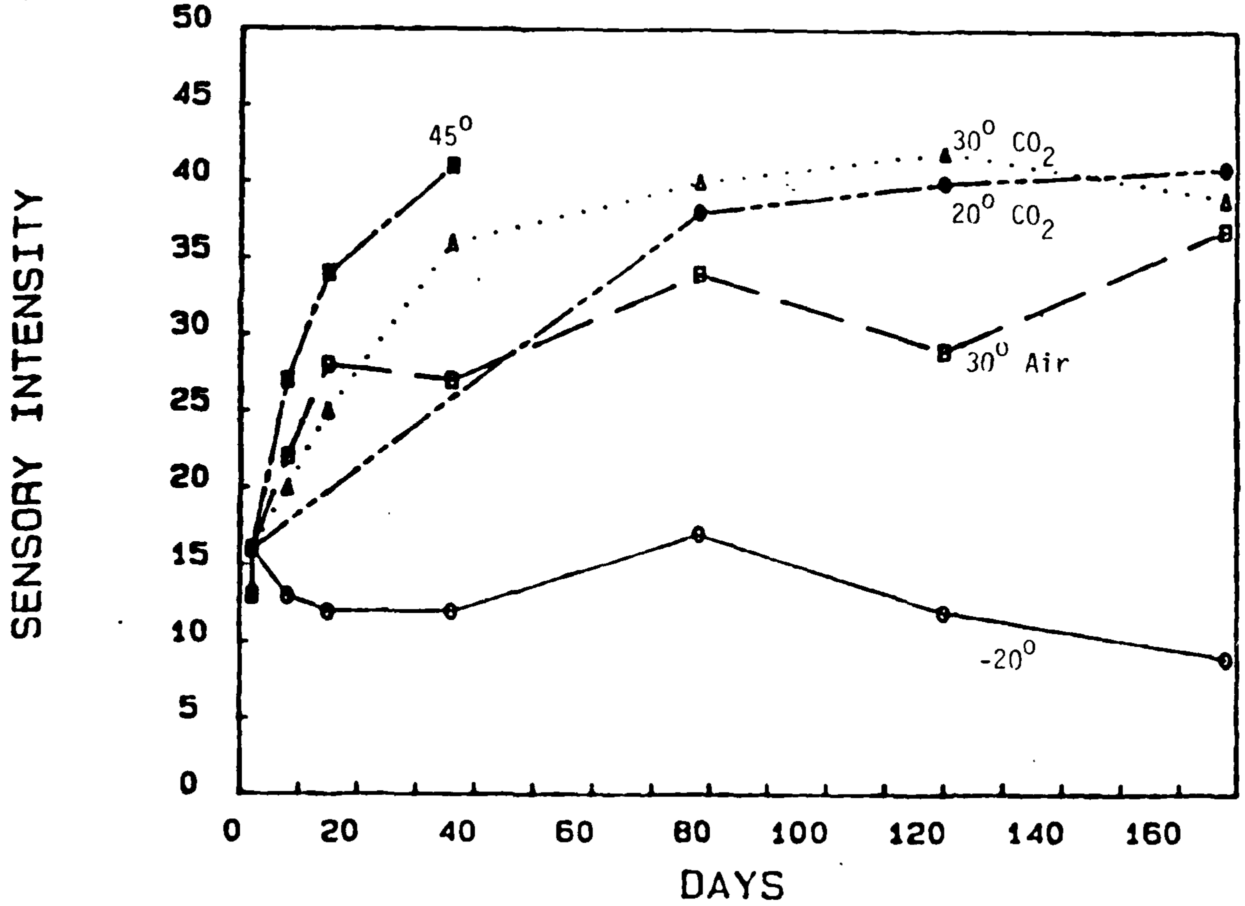

Figure 30. Effect of storage conditions on dryness (texture) of humectant bread (7-1-2).

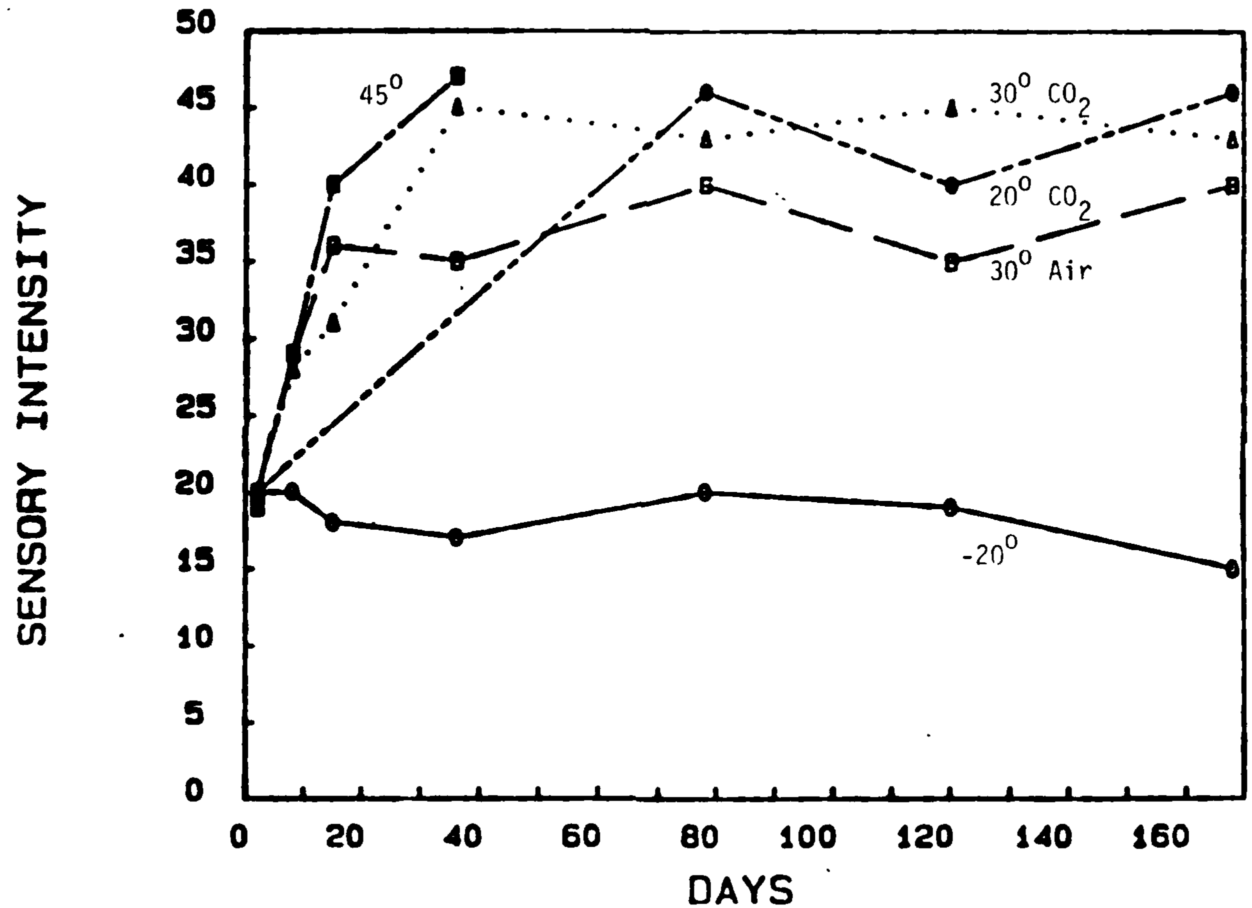

Figure 31. Effect of storage conditions on firmness of humectant bread (7-1-2).

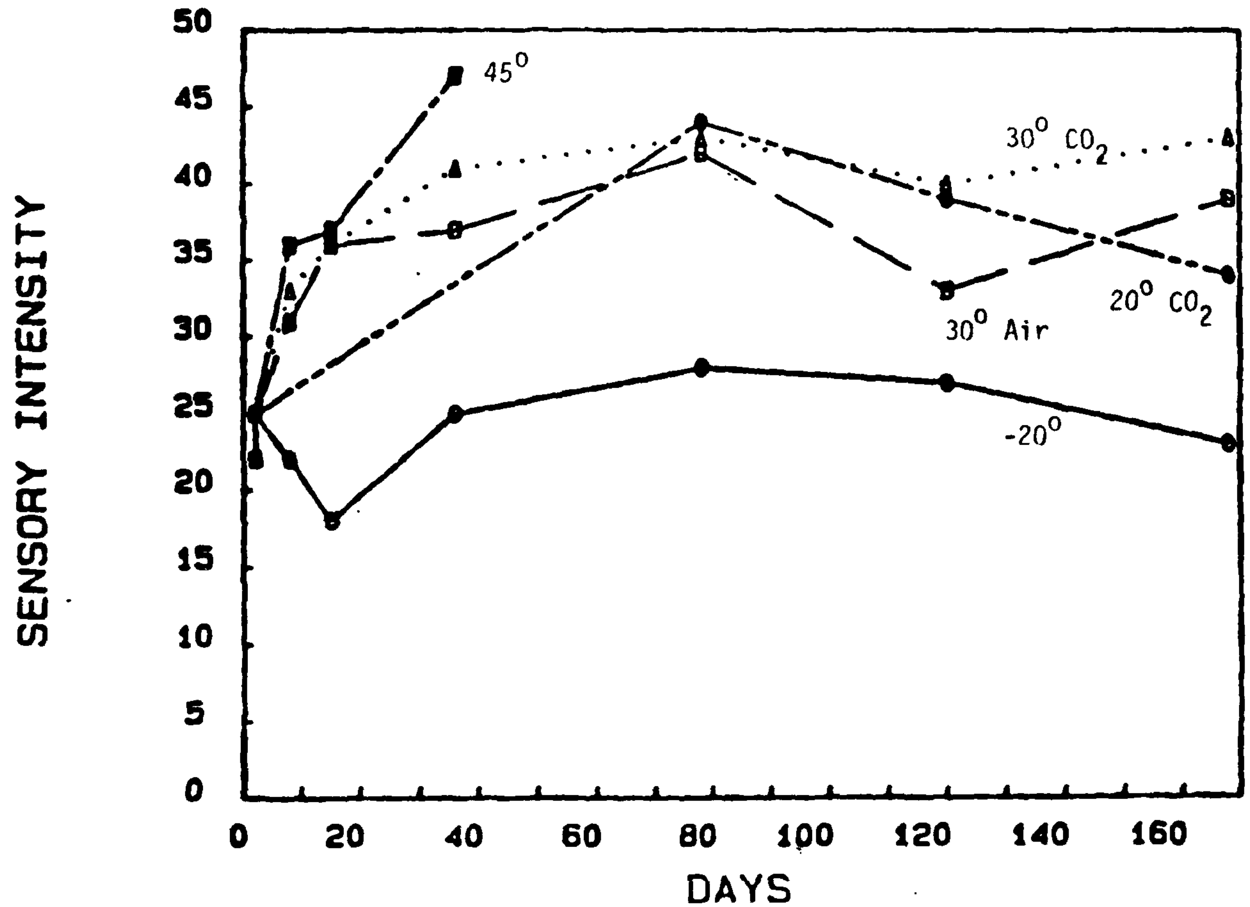

Figure 32. Effect of storage conditions on springiness of humectant bread (7-1-2).

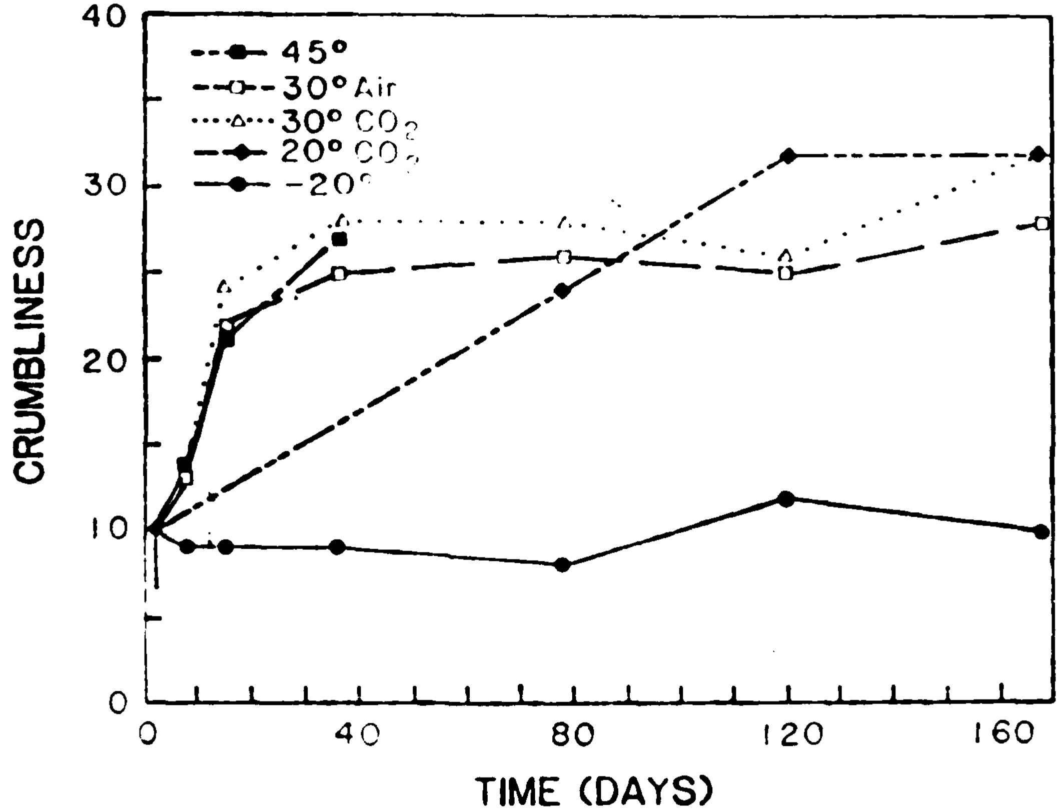

Figure 33. Effect of storage conditions on crumbliness of humectant bread (7-1-2).

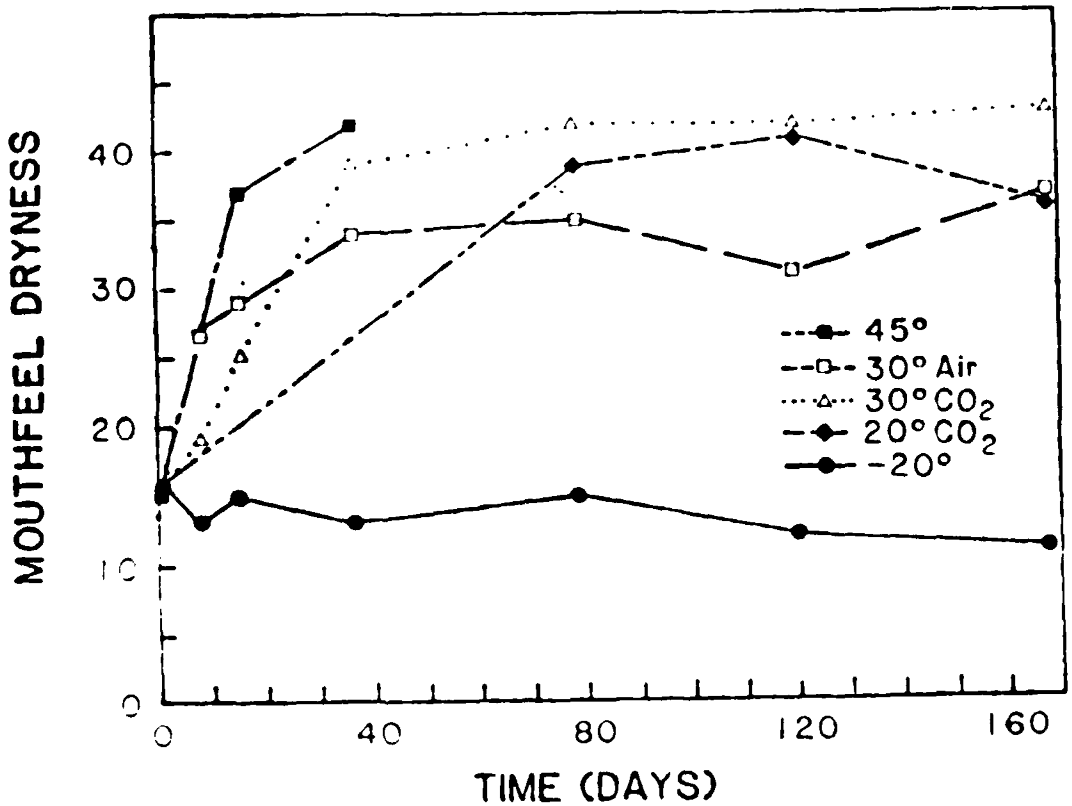

Figure 34. Effect of storage conditions on dryness (mouthfeel) of humectant bread (7-1-2).

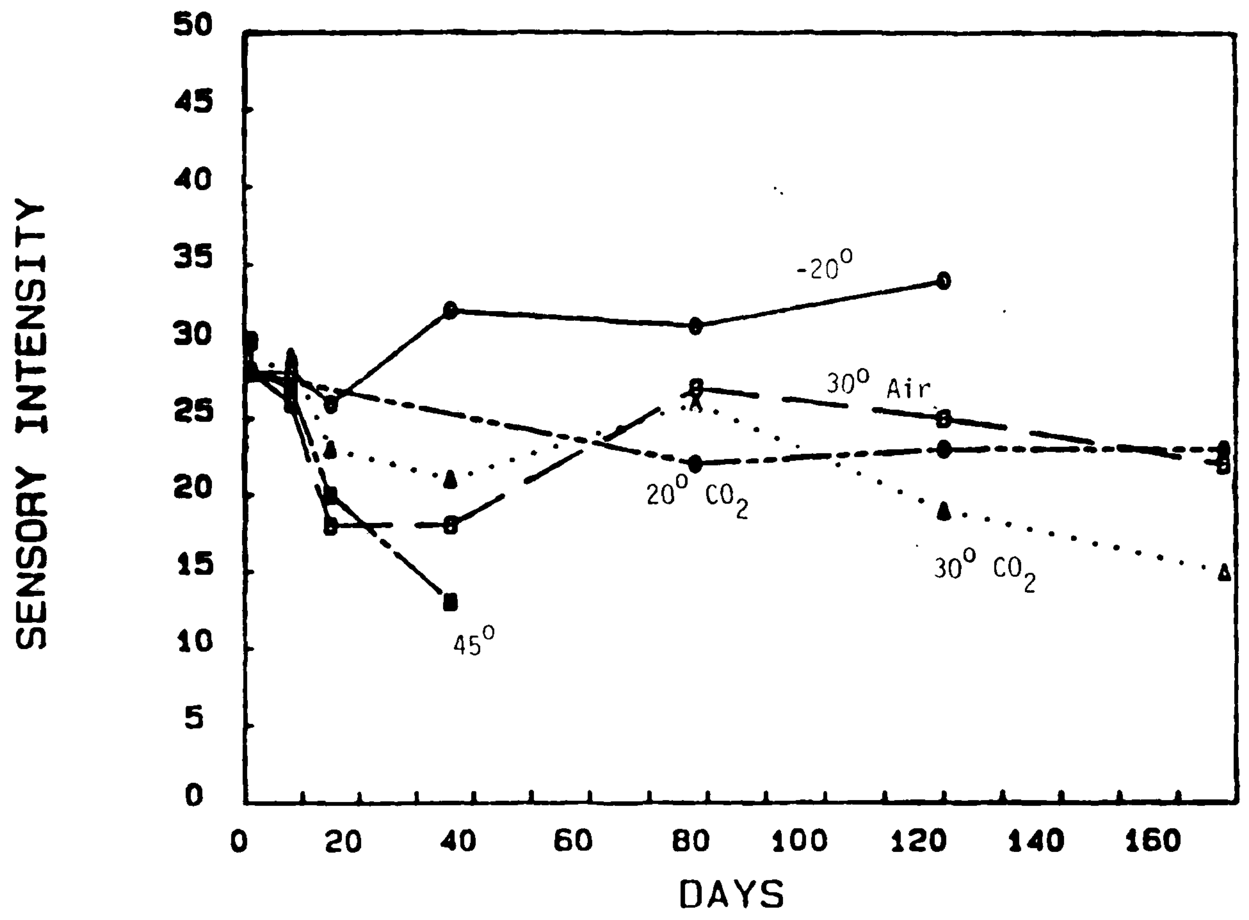

Figure 35. Effect of storage conditions on gumminess of humectant bread (7-1-2).

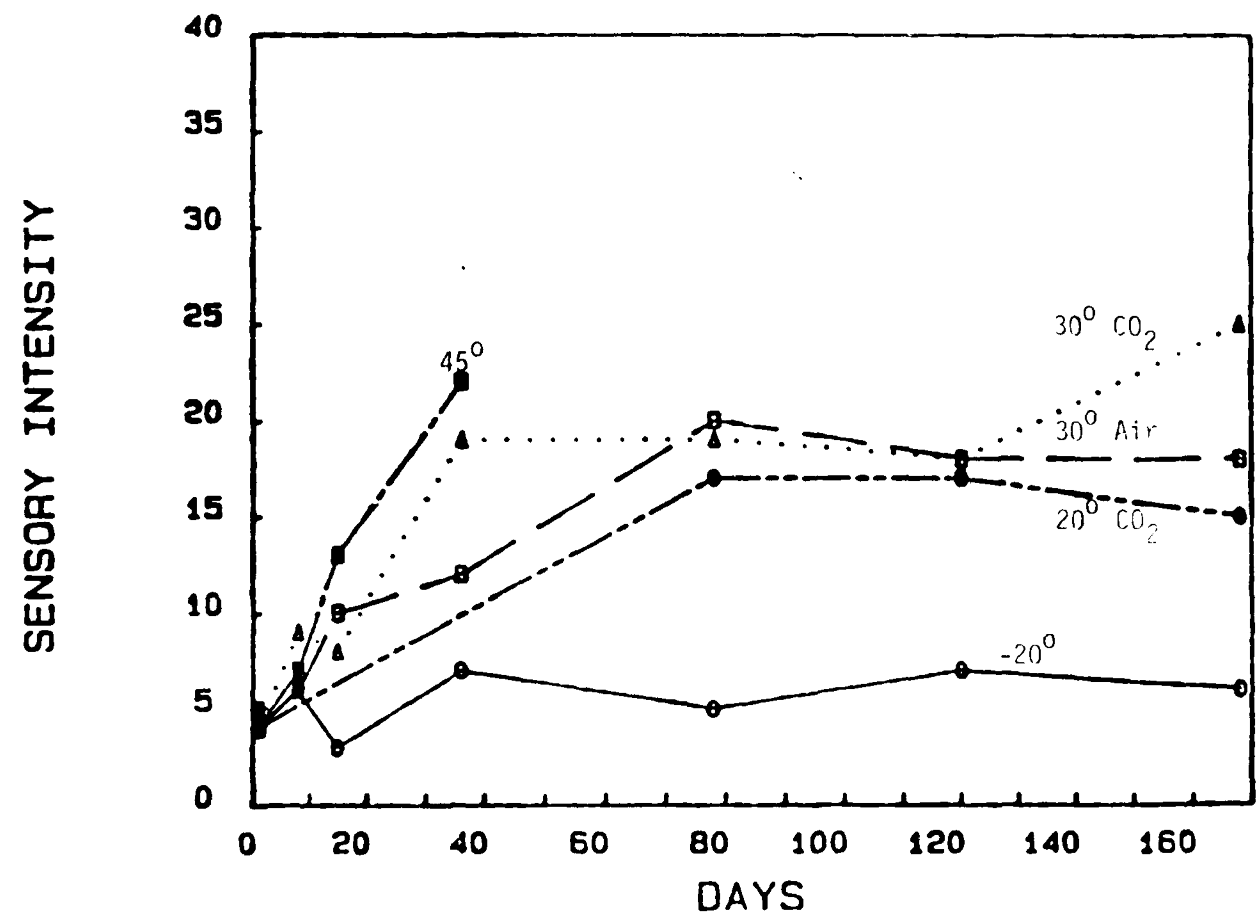

Figure 36. Effect of storage conditions on graininess of humectant bread (7-1-2).

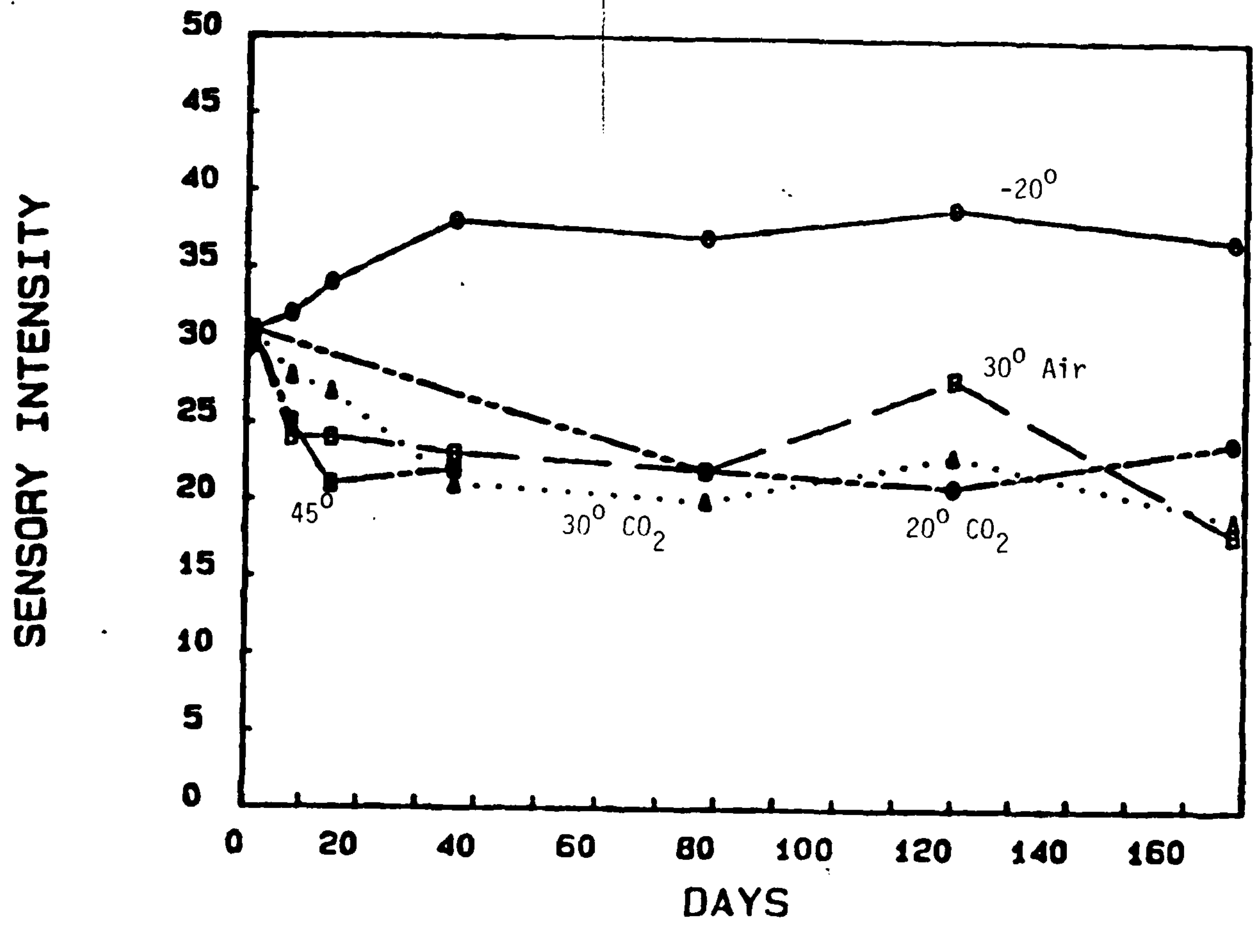

Figure 37. Effect of storage conditions on rate of hydration of humectant bread (7-1-2).

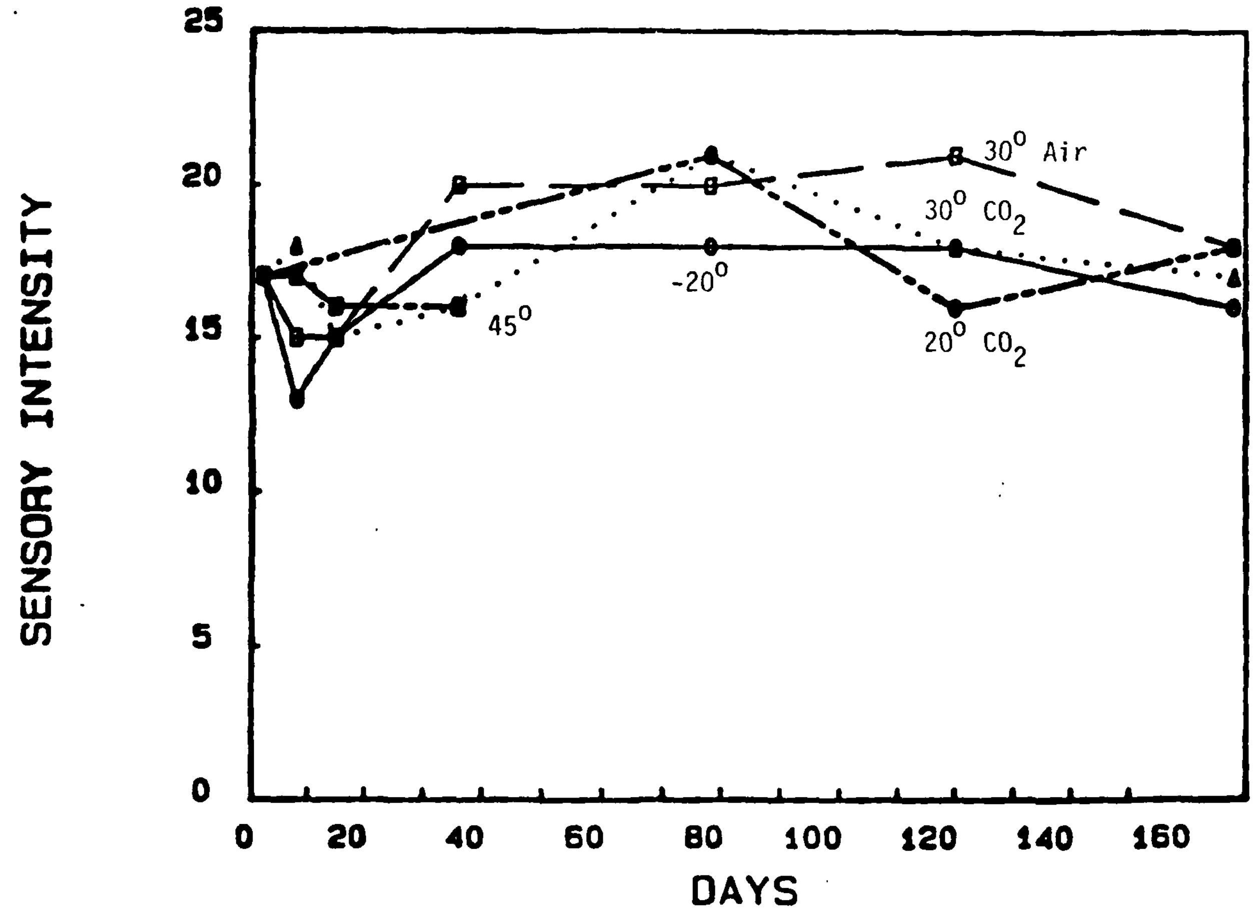

Figure 38. Effect of storage conditions on sweet flavor of crumb in humectant bread (7-1-2).

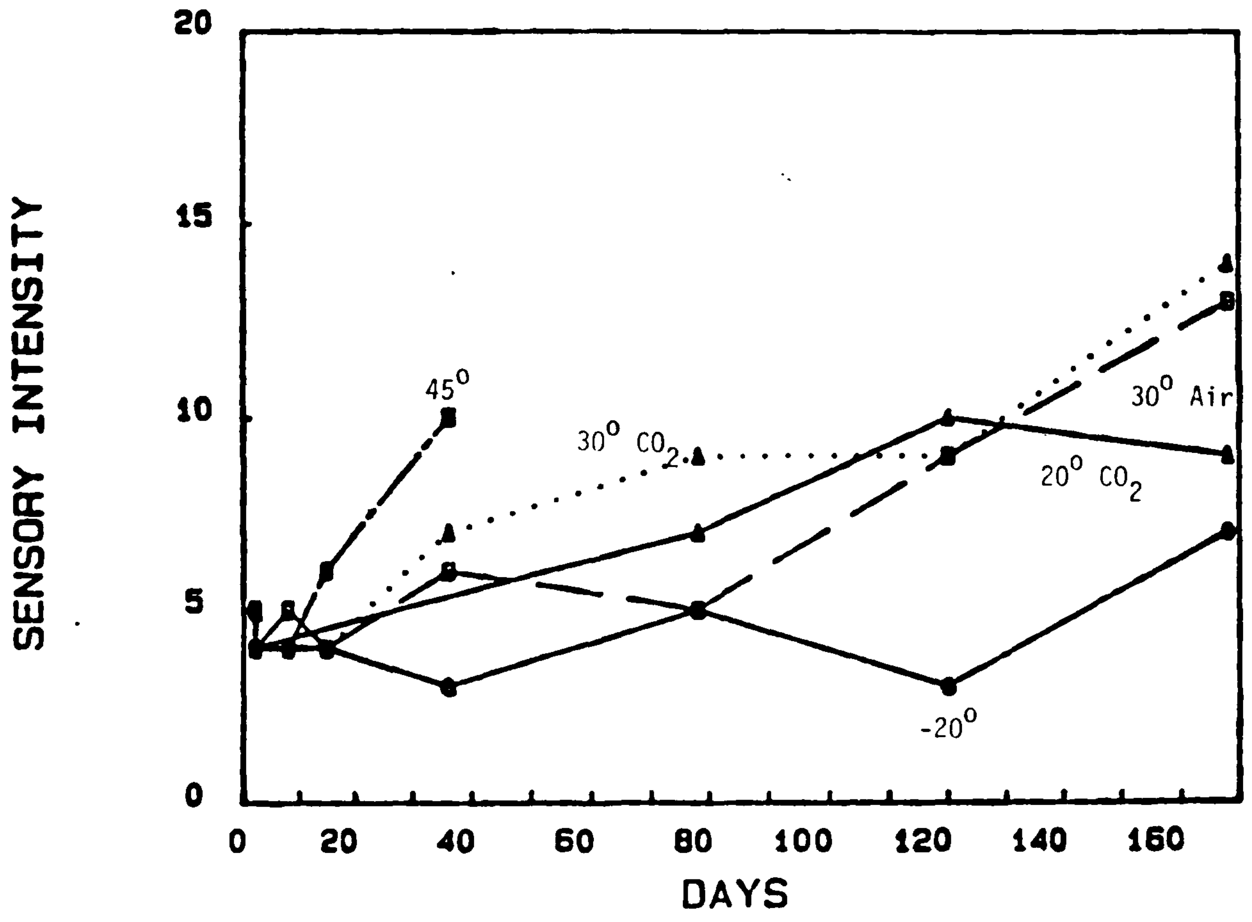

Figure 39. Effect of storage conditions on sour flavor of crumb in humectant bread (7-1-2).

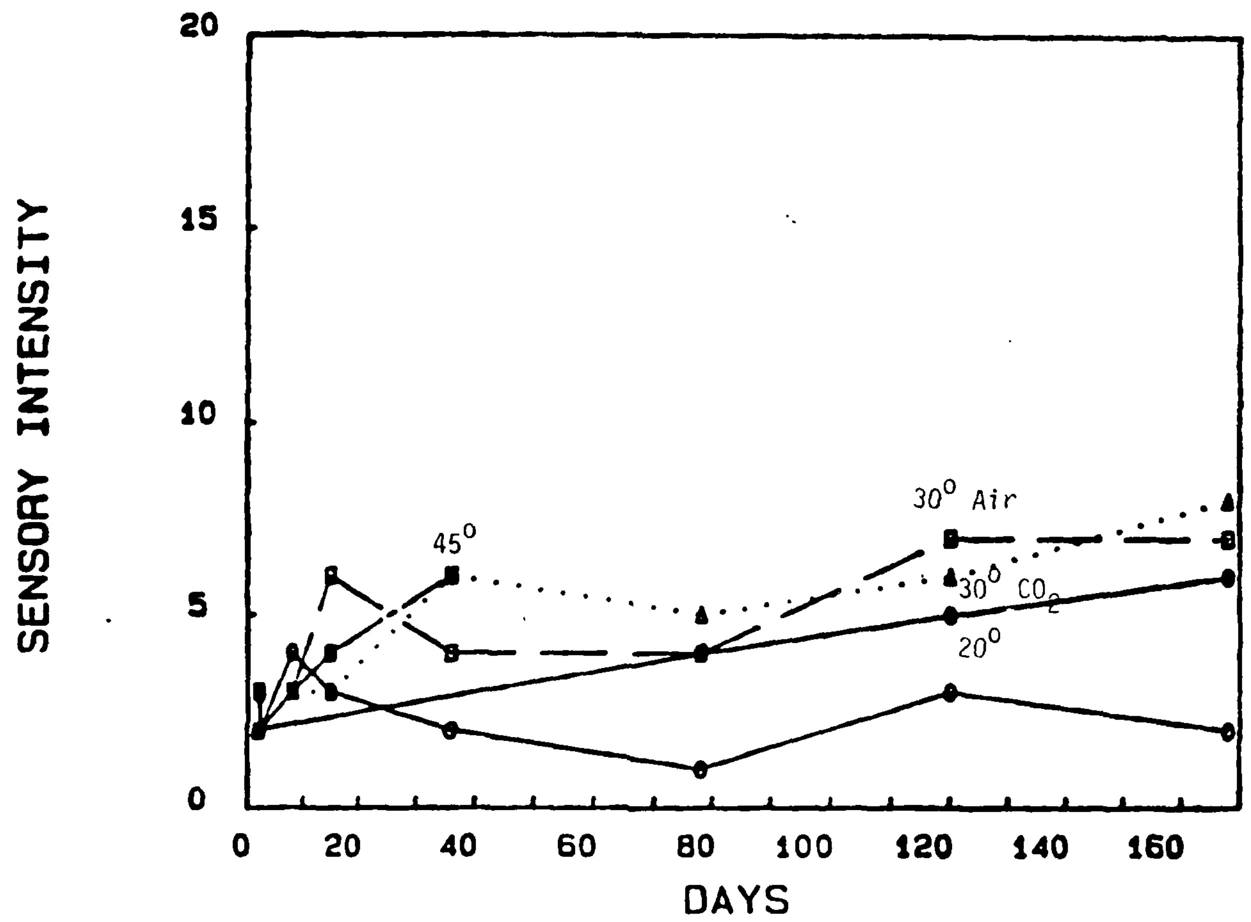

Figure 40. Effect of storage conditions on bitter flavor of crumb in humectant bread (?-1-2).

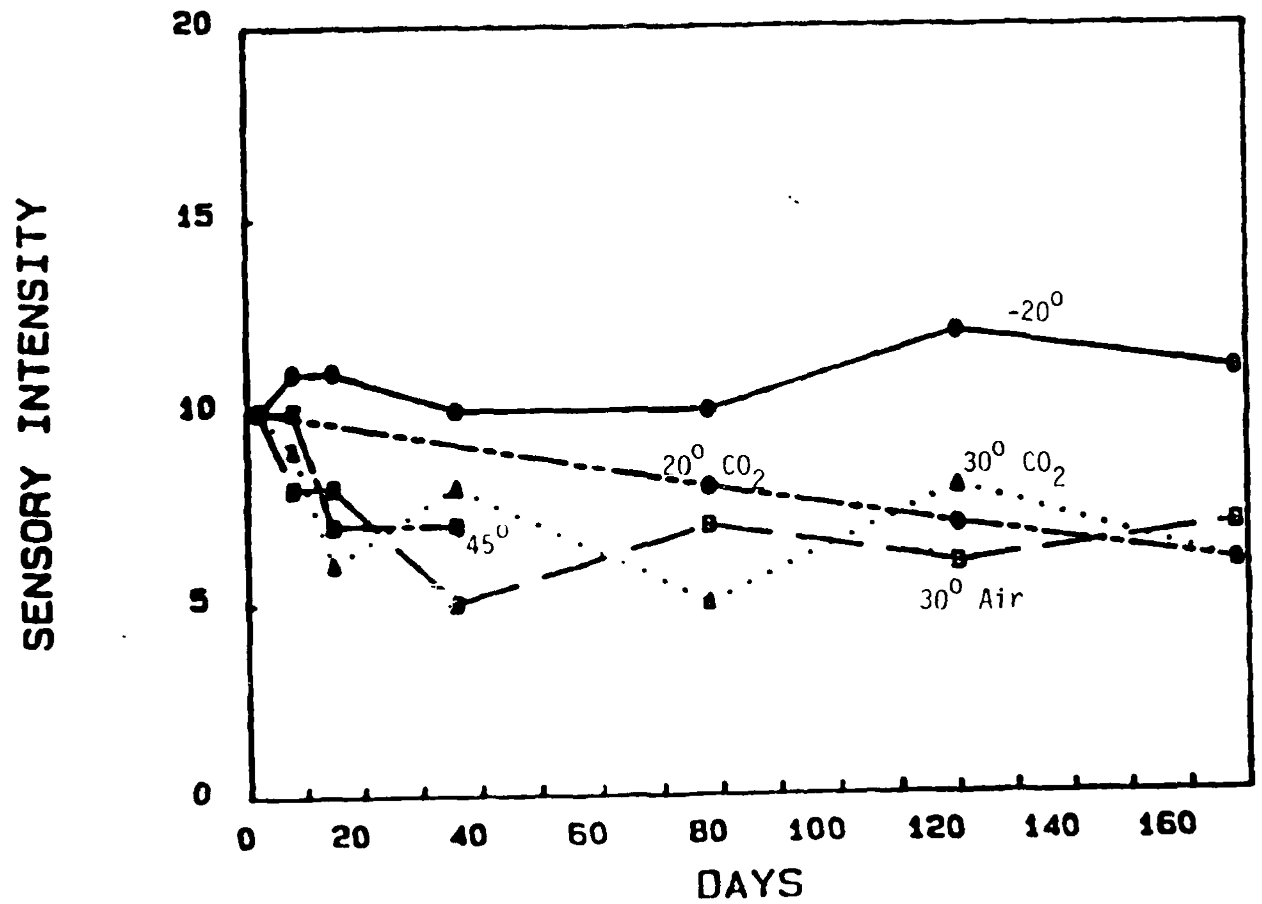

Figure 41. Effect of storage conditions on yeasty flavor of crumb in humectant bread (7-1-2).

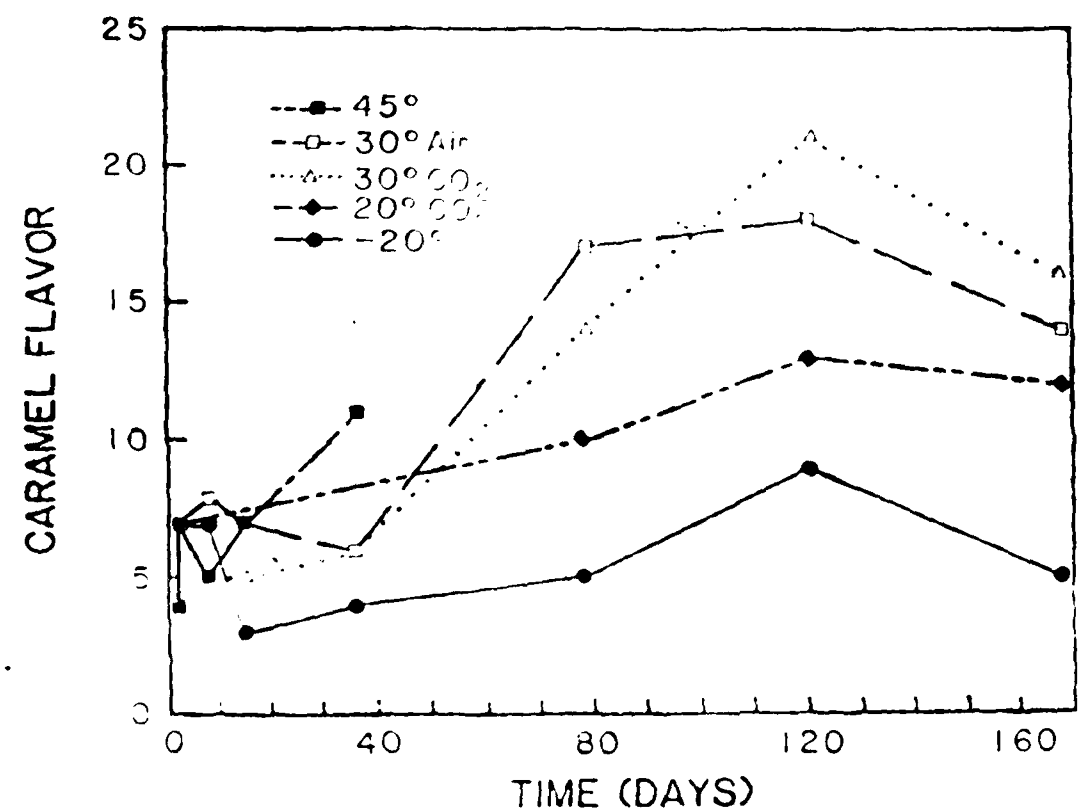

Figure 42. Effect of storage conditions on caramel flavor of crumb in humectant bread (7-1-2).

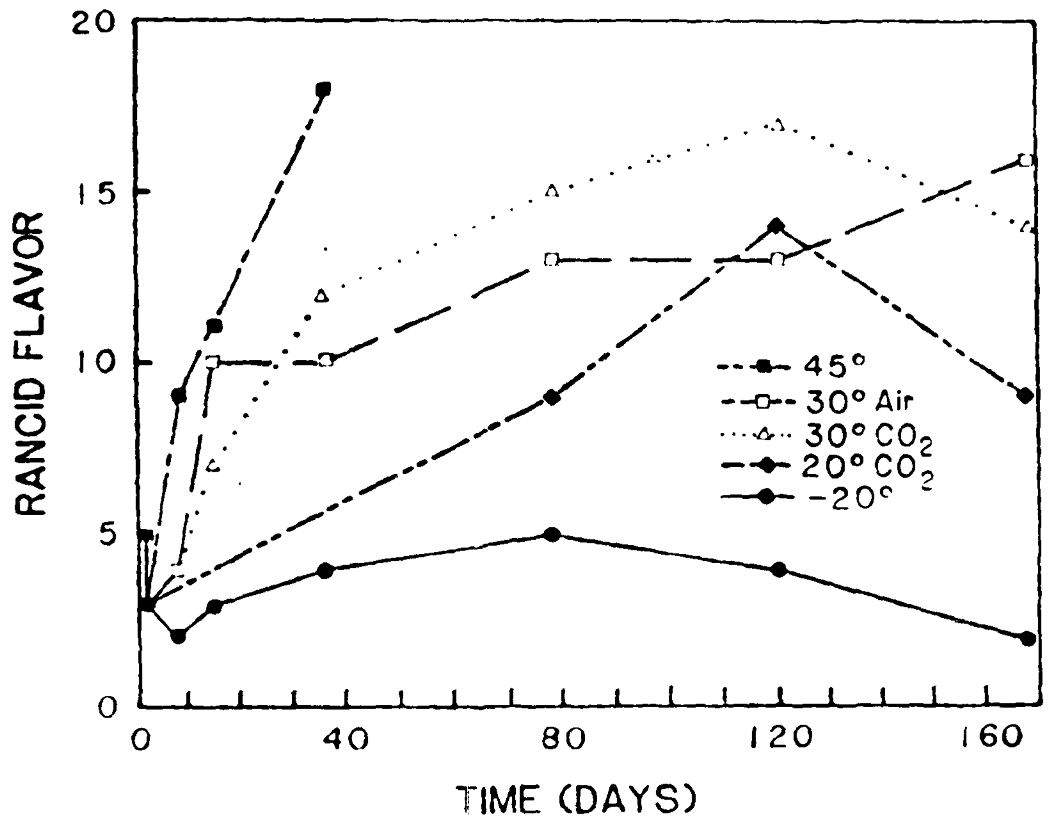

Figure 43. Effect of storage conditions on rancid flavor of crumb in humectant bread (7-1-2).

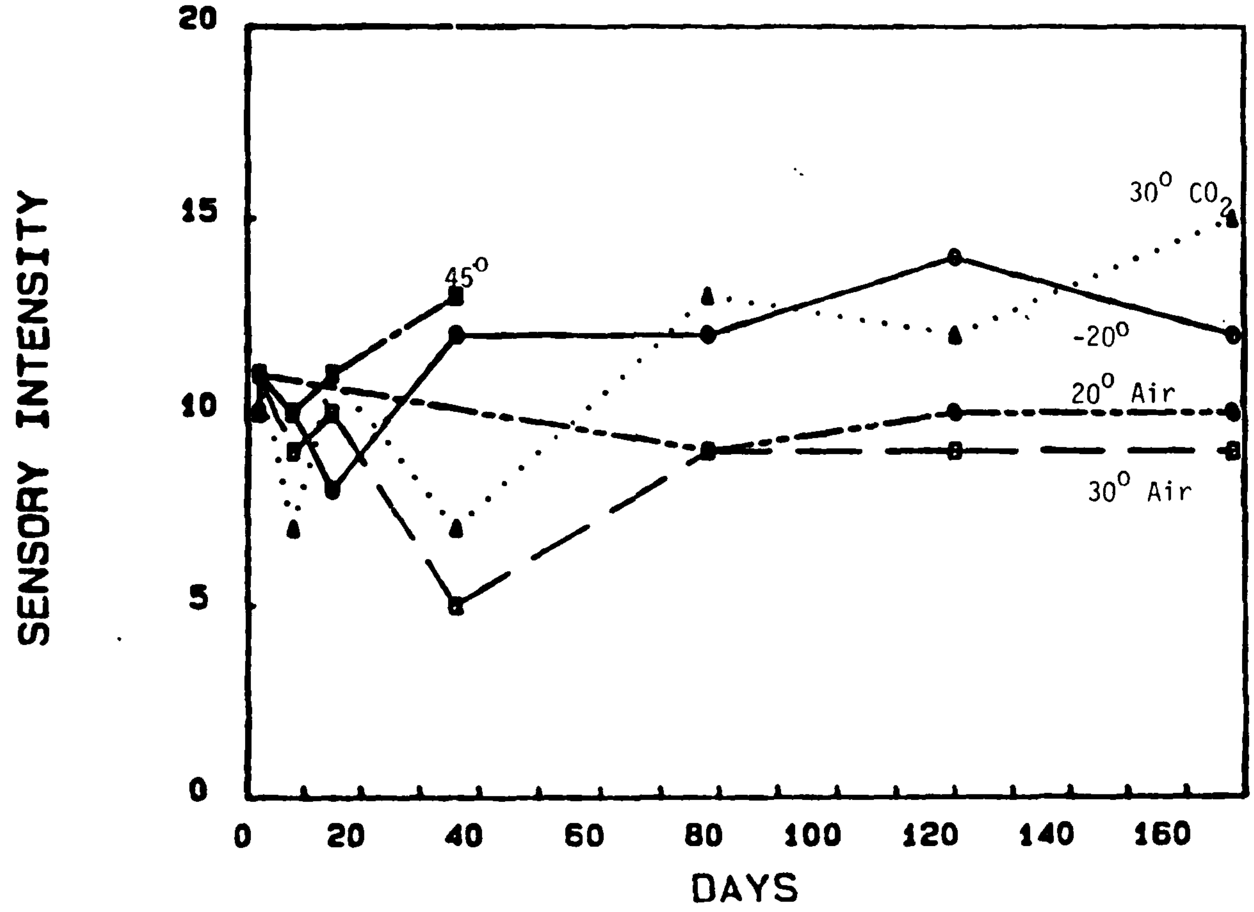

Figure 44. Effect of storage conditions on burnt flavor of crust of humectant bread (7-1-2).

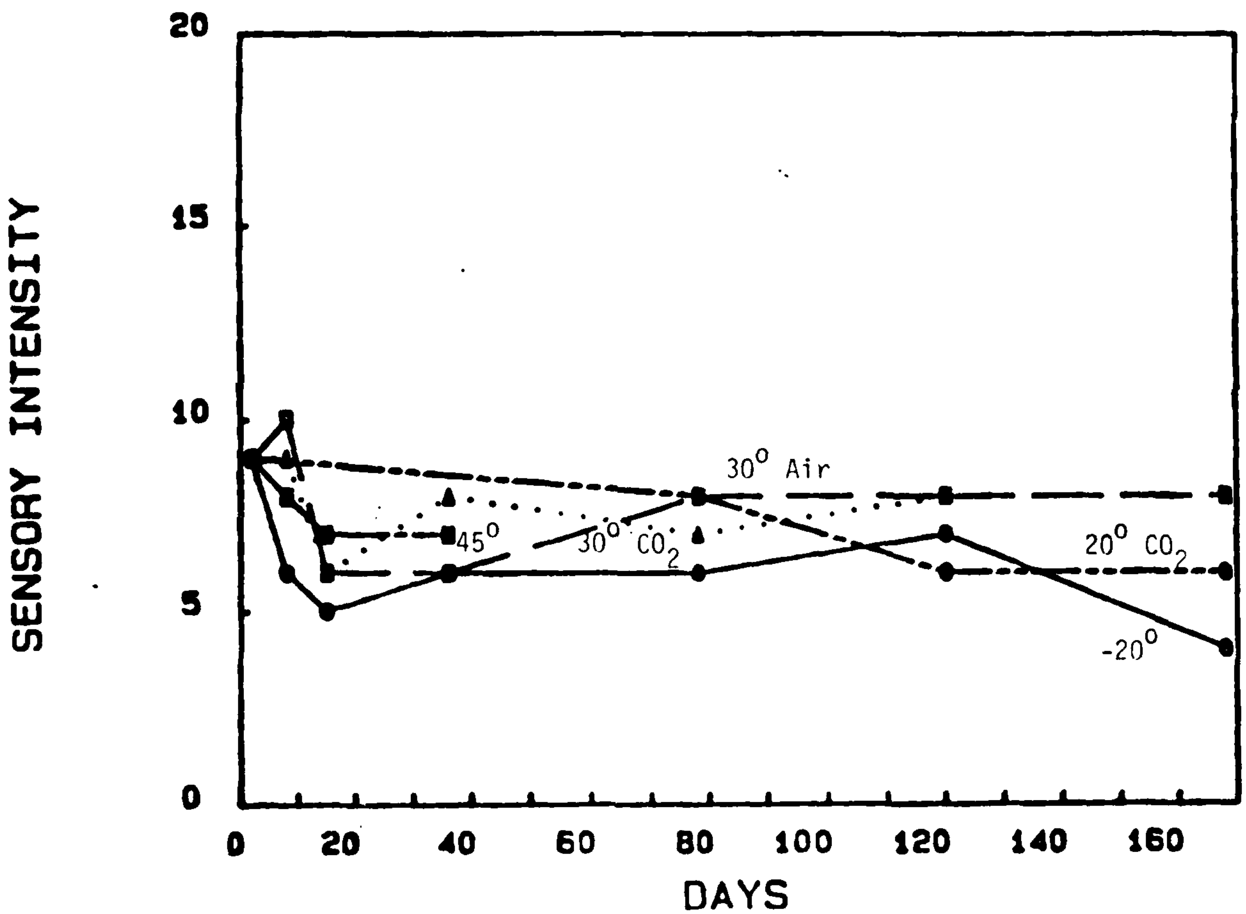

Figure 45. Effect of storage conditions on bitter flavor of crust of humectant bread (7-1-2).

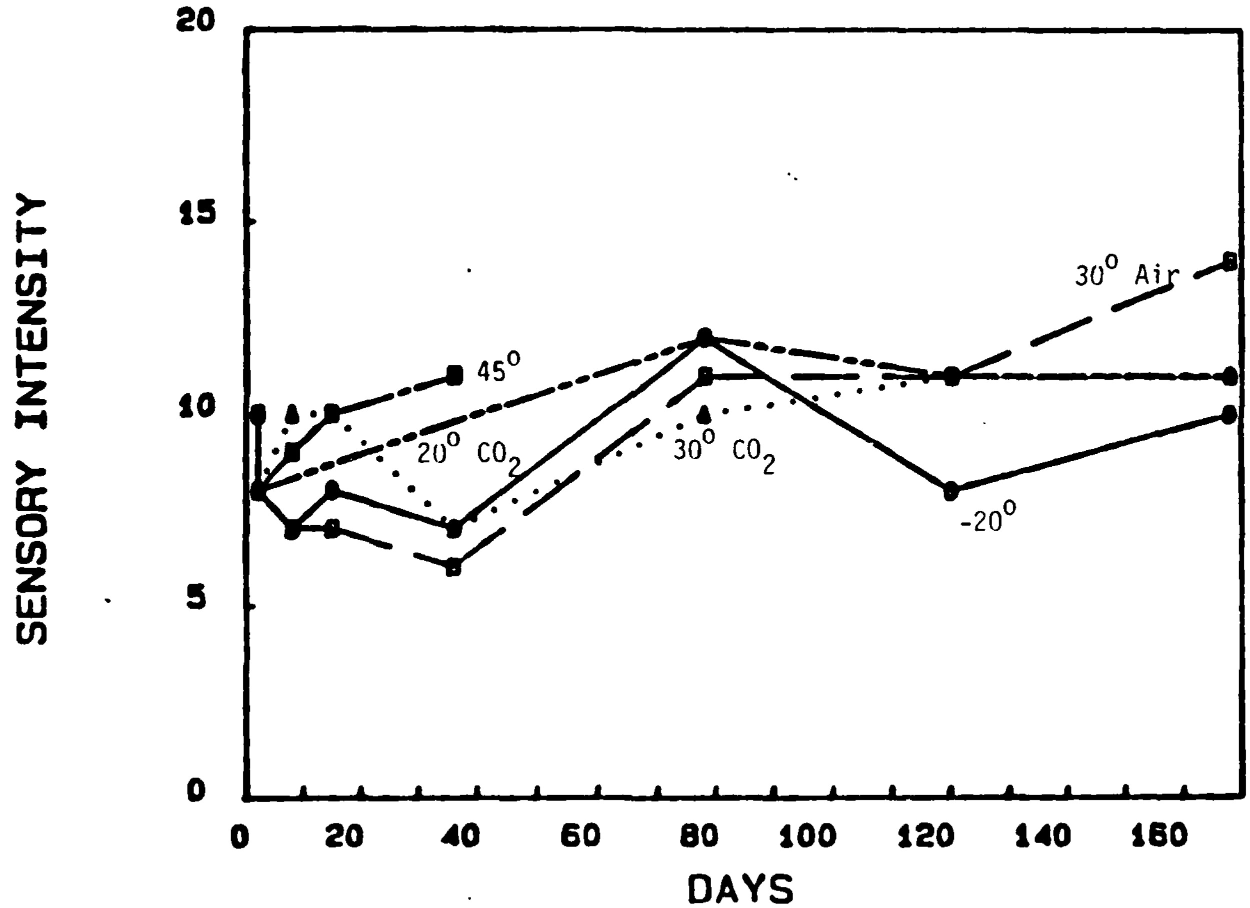

Figure 46. Effect of storage conditions on sweet flavor of crust of humectant bread (7-1-2).

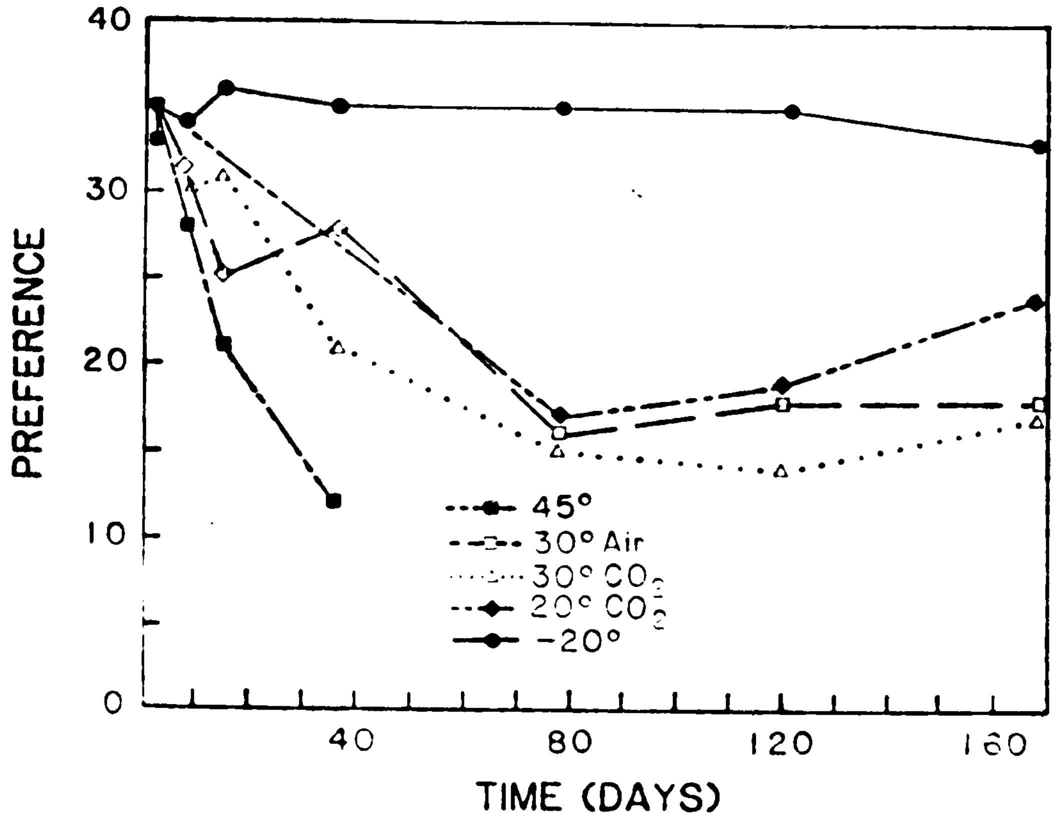

Figure 47. Effect of storage conditions on overall preference of humectant bread (7-1-2).

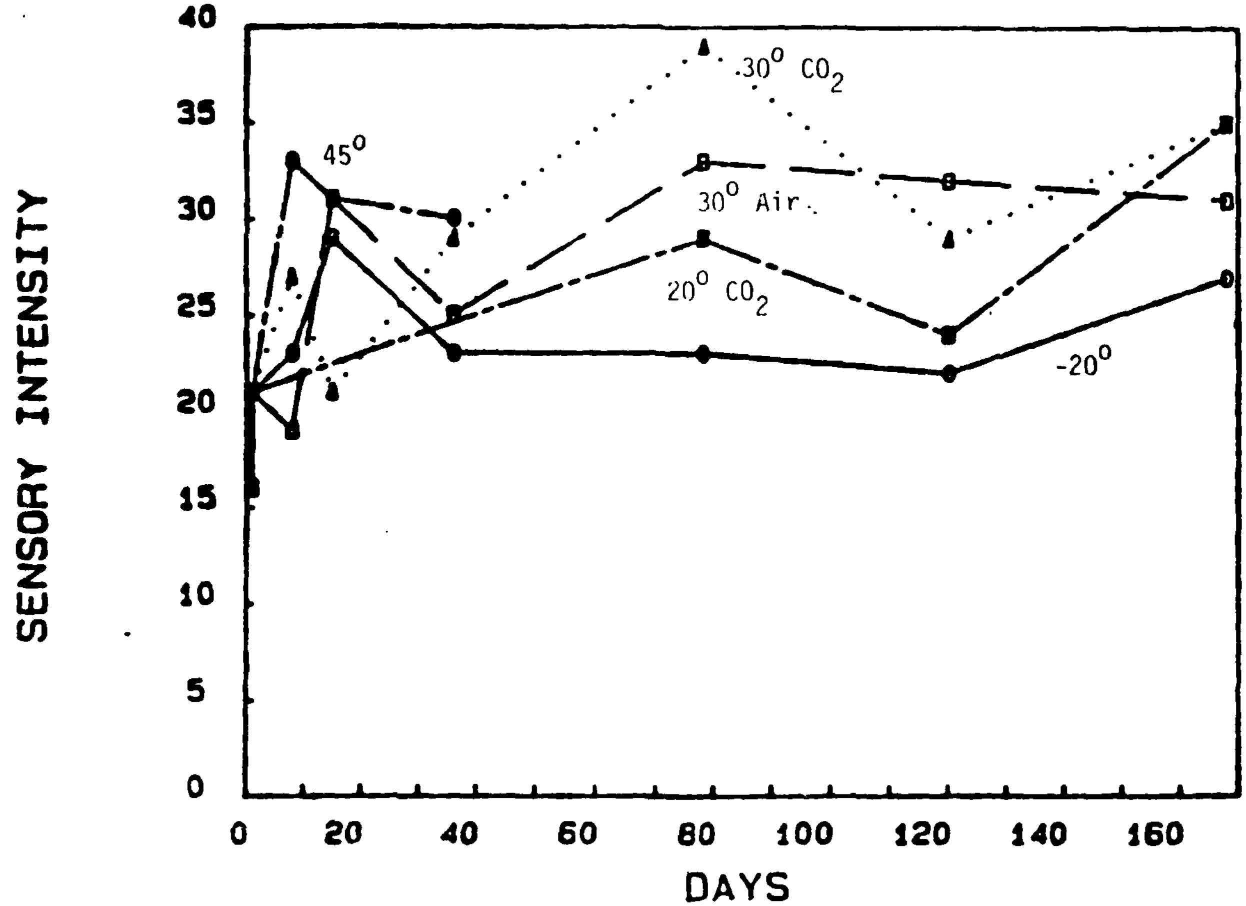

Figure 48. Effect of storage conditions on crust browning of humectant bread (6-1.5-2).

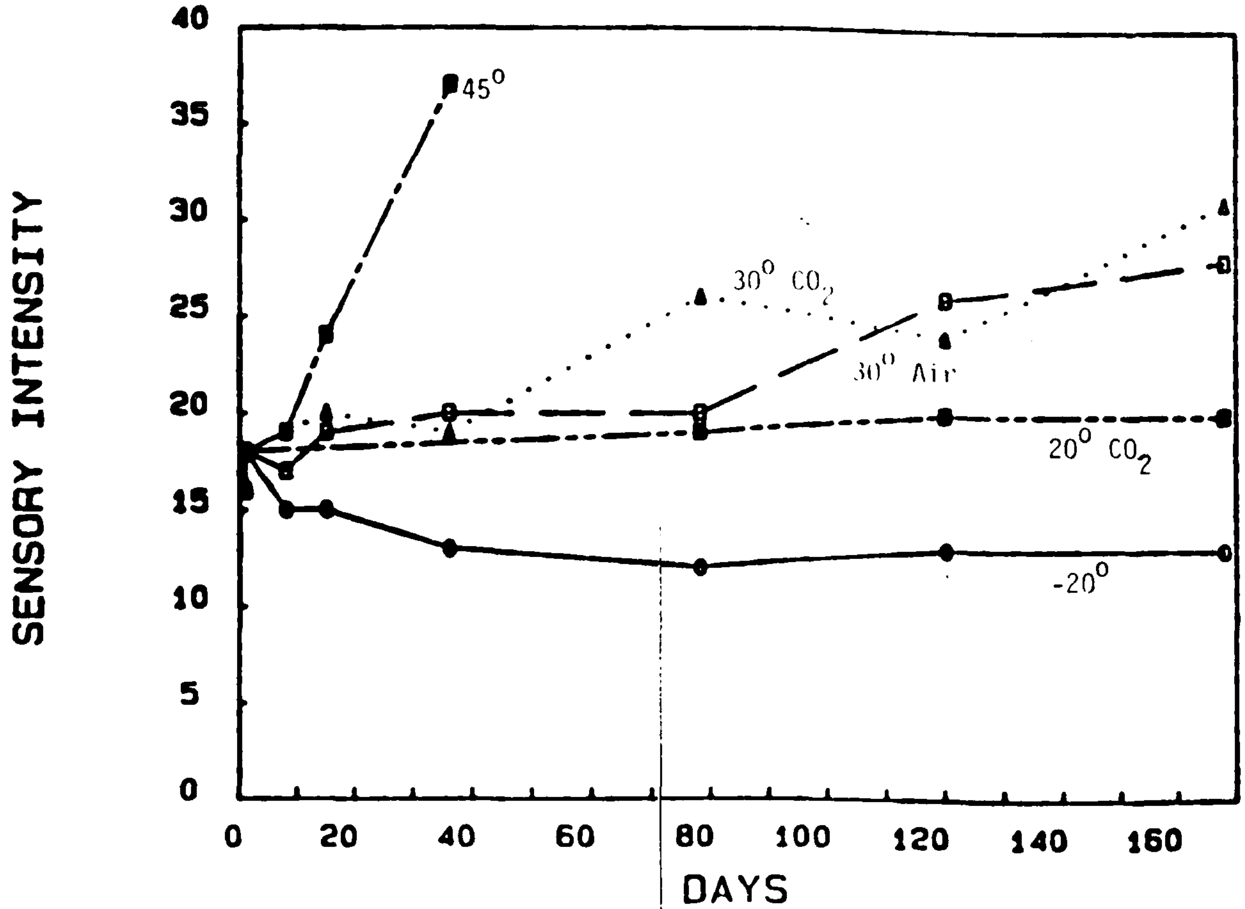

Figure 49. Effect of storage conditions on crumb color of humectant bread (6-1.5-2).

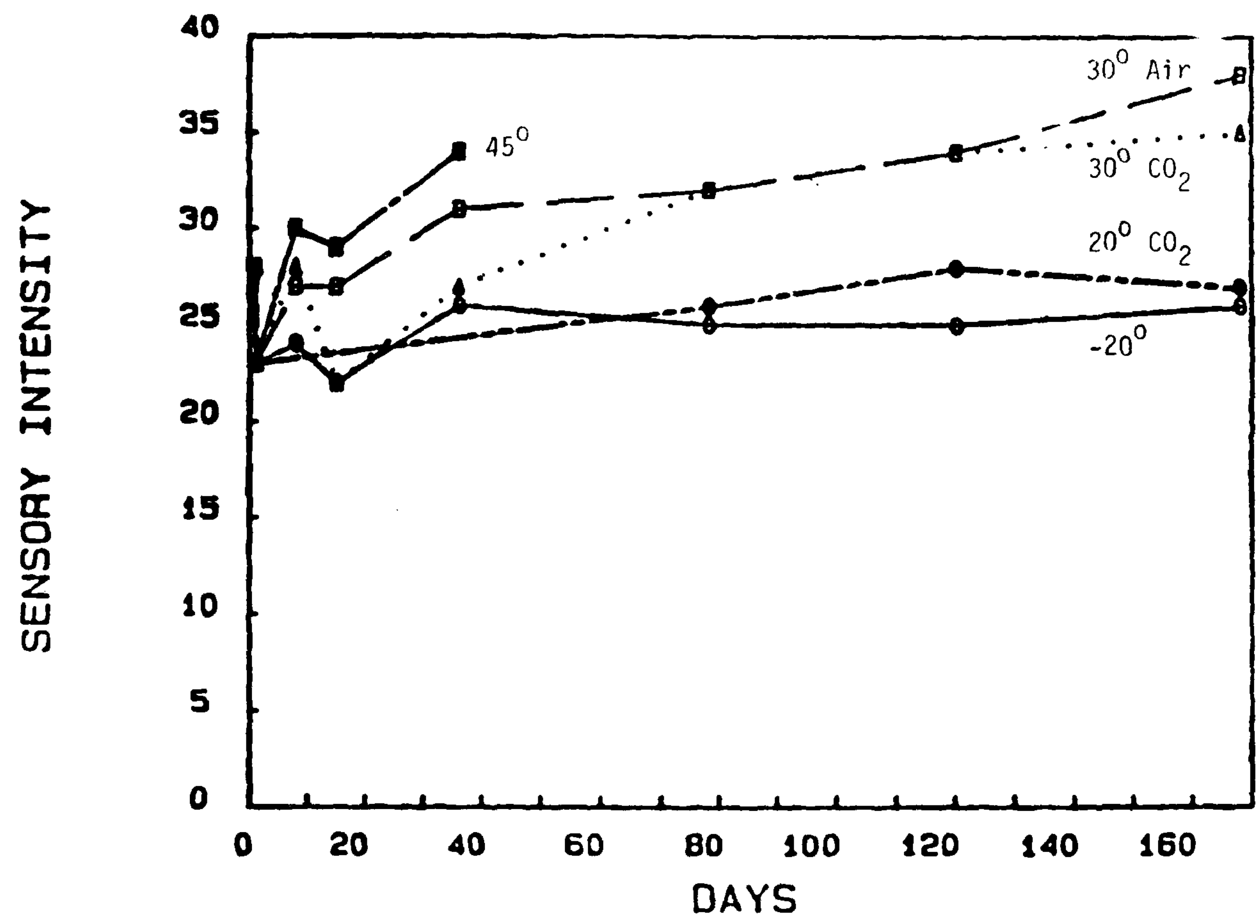

Figure 50. Effect of storage conditions on overall aroma intensity of humectant bread (6-1.5-2).

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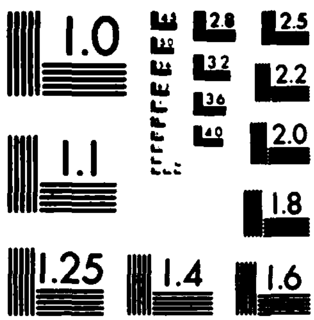

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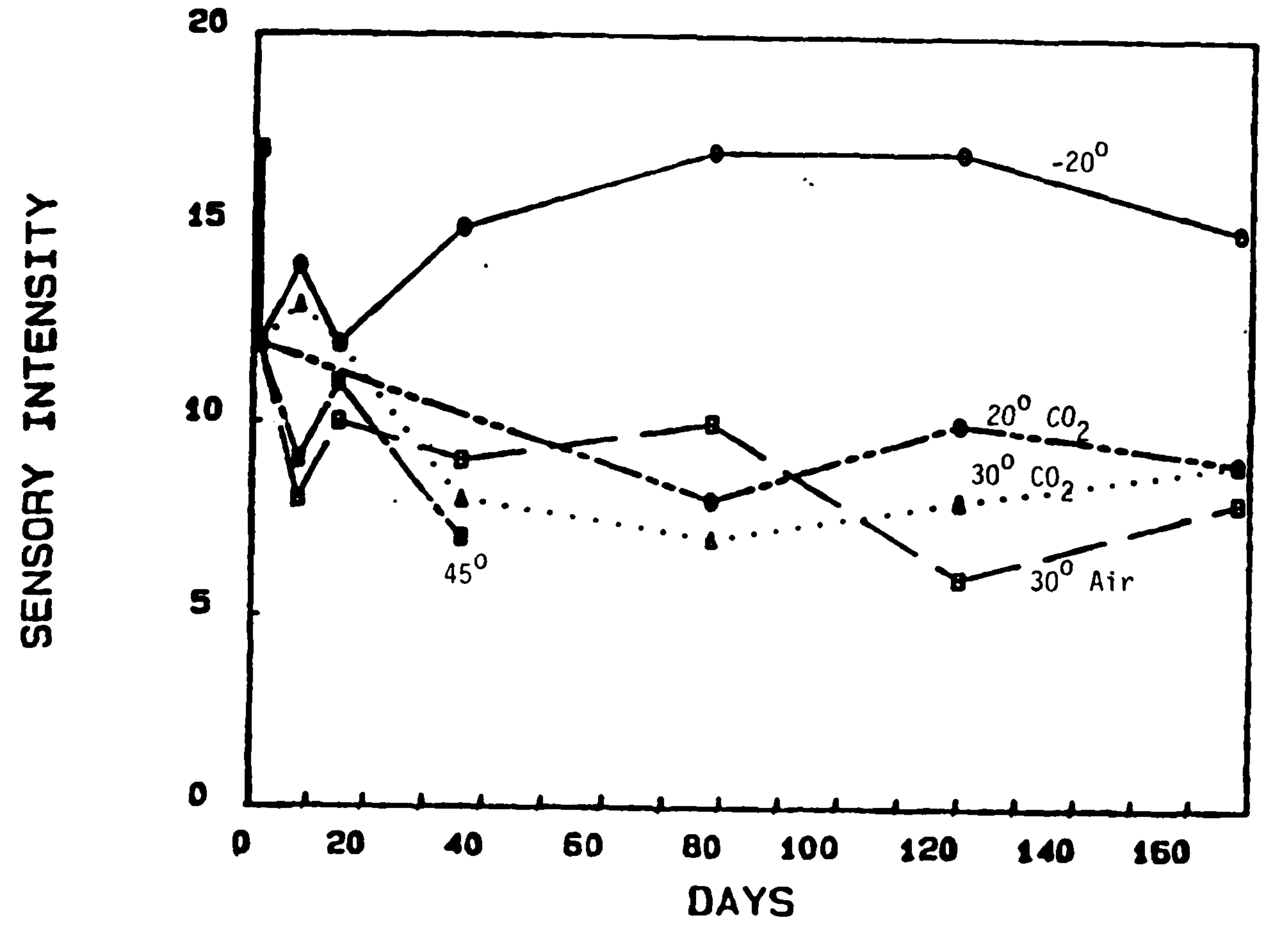

Figure 51. Effect of storage conditions on yeasty aroma of humectant bread (6-1.5-2).

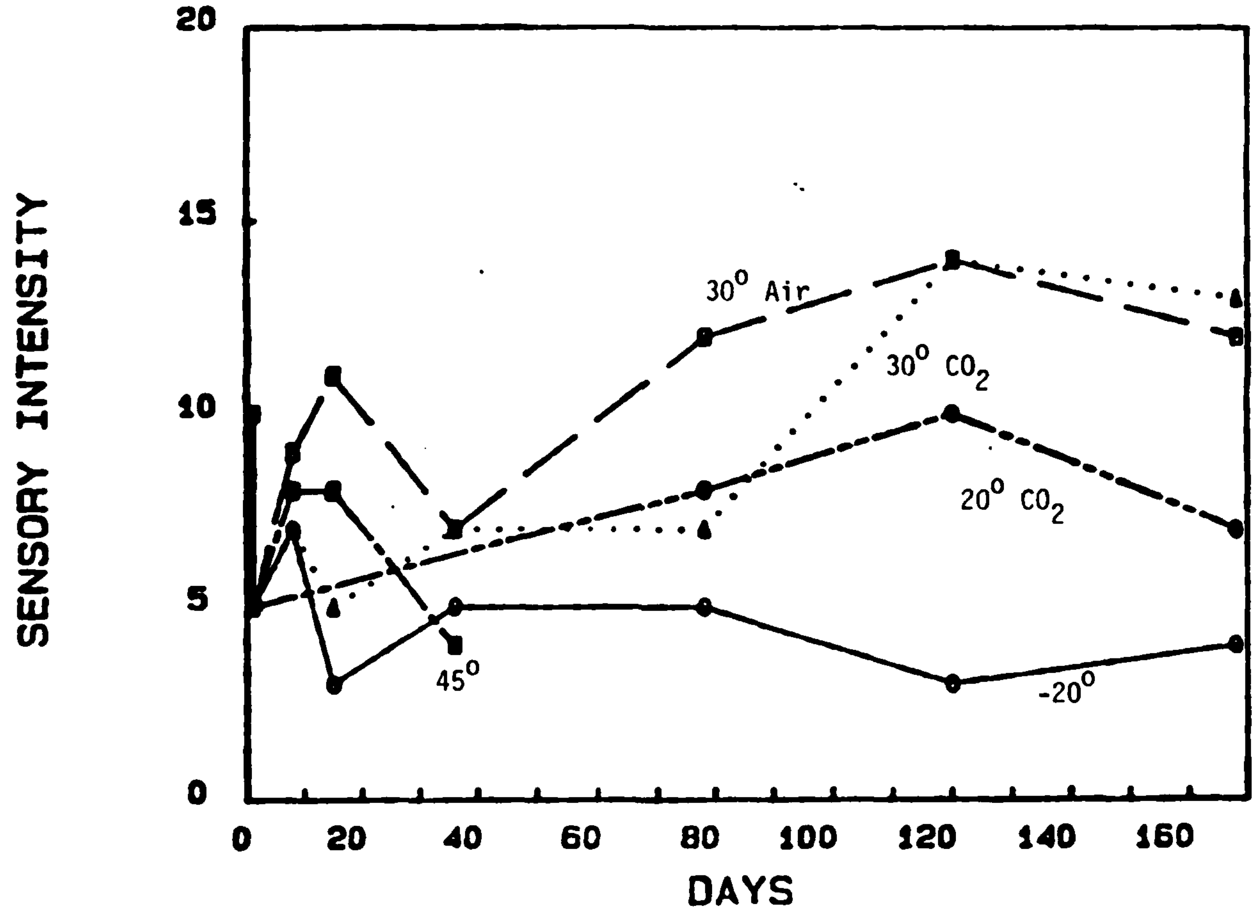

Figure 52. Effect of storage conditions on acrid aroma of humectant bread (6-1.5-2).

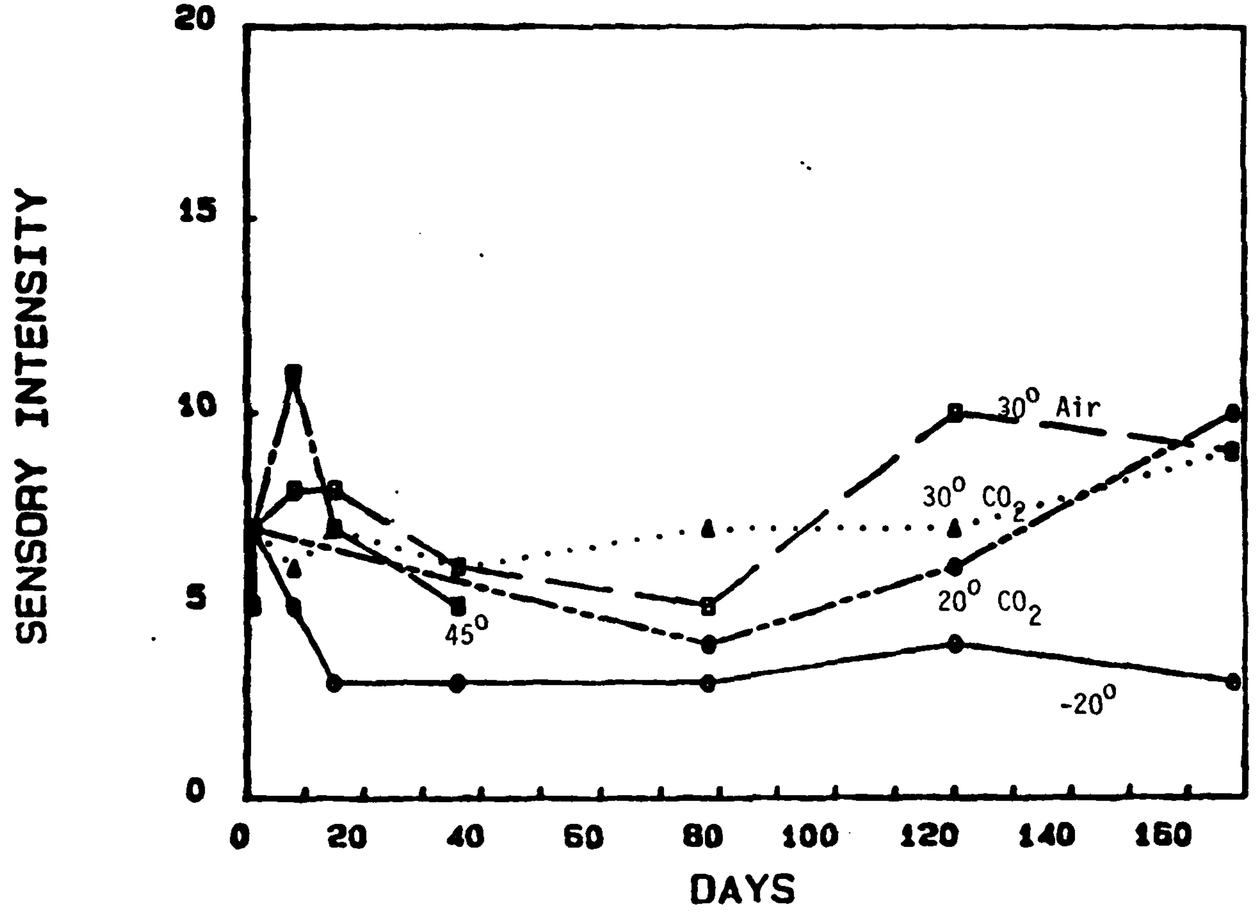

Figure 53. Effect of storage conditions on sour aroma of humectant bread (6-1.5-2).

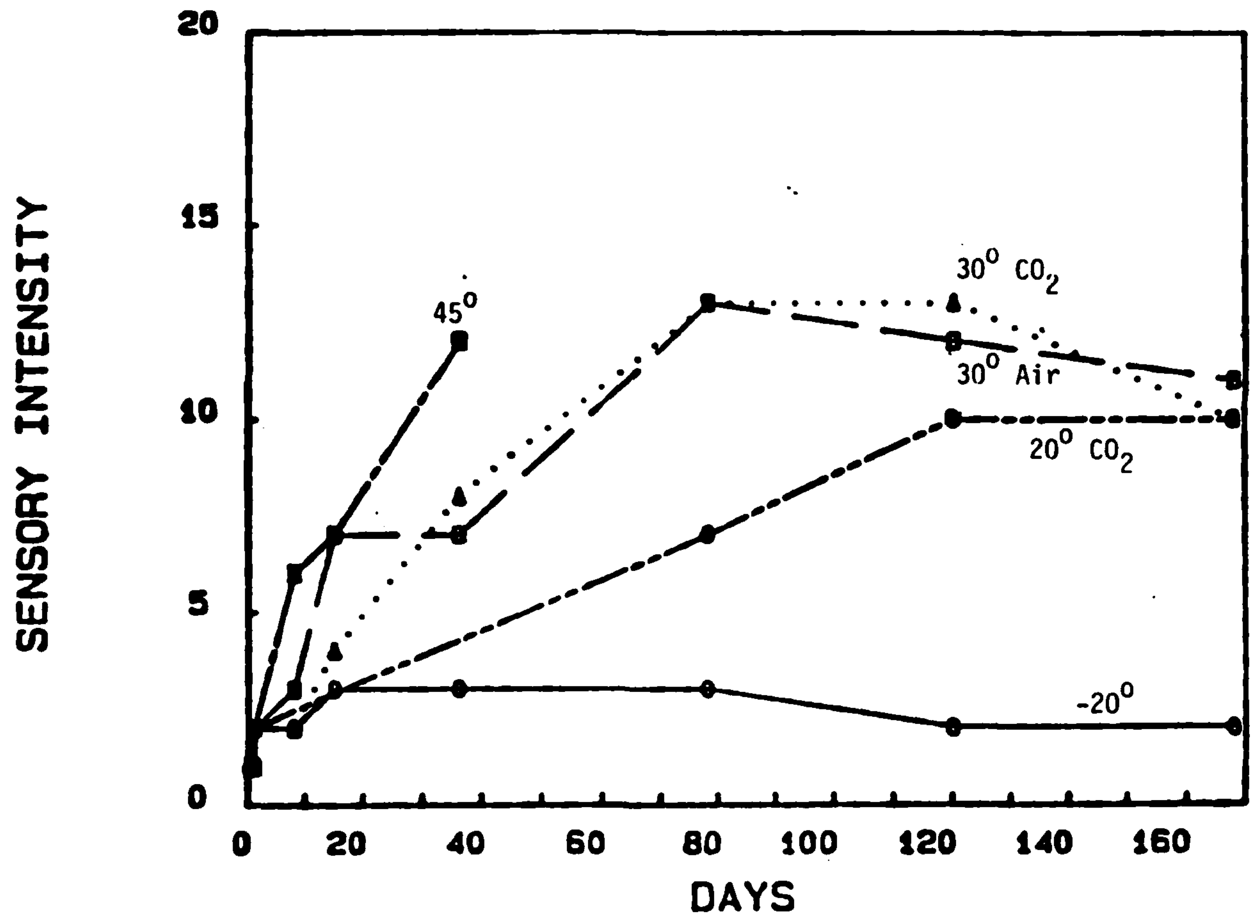

Figure 54. Effect of storage conditions on rancid aroma of humectant bread (6-1.5-2).

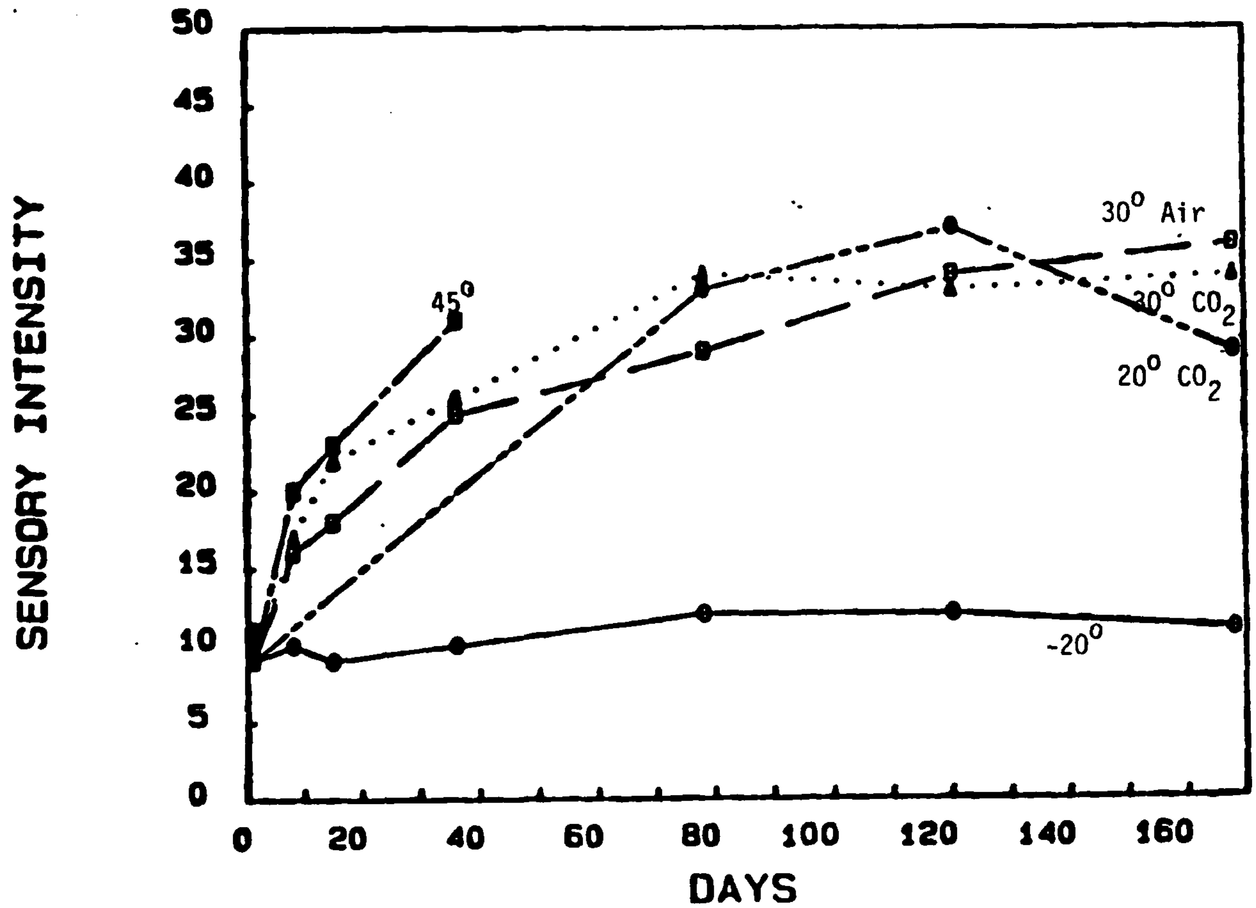

Figure 55. Effect of storage conditions on dryness (texture) of humectant bread (6-1.5-2).

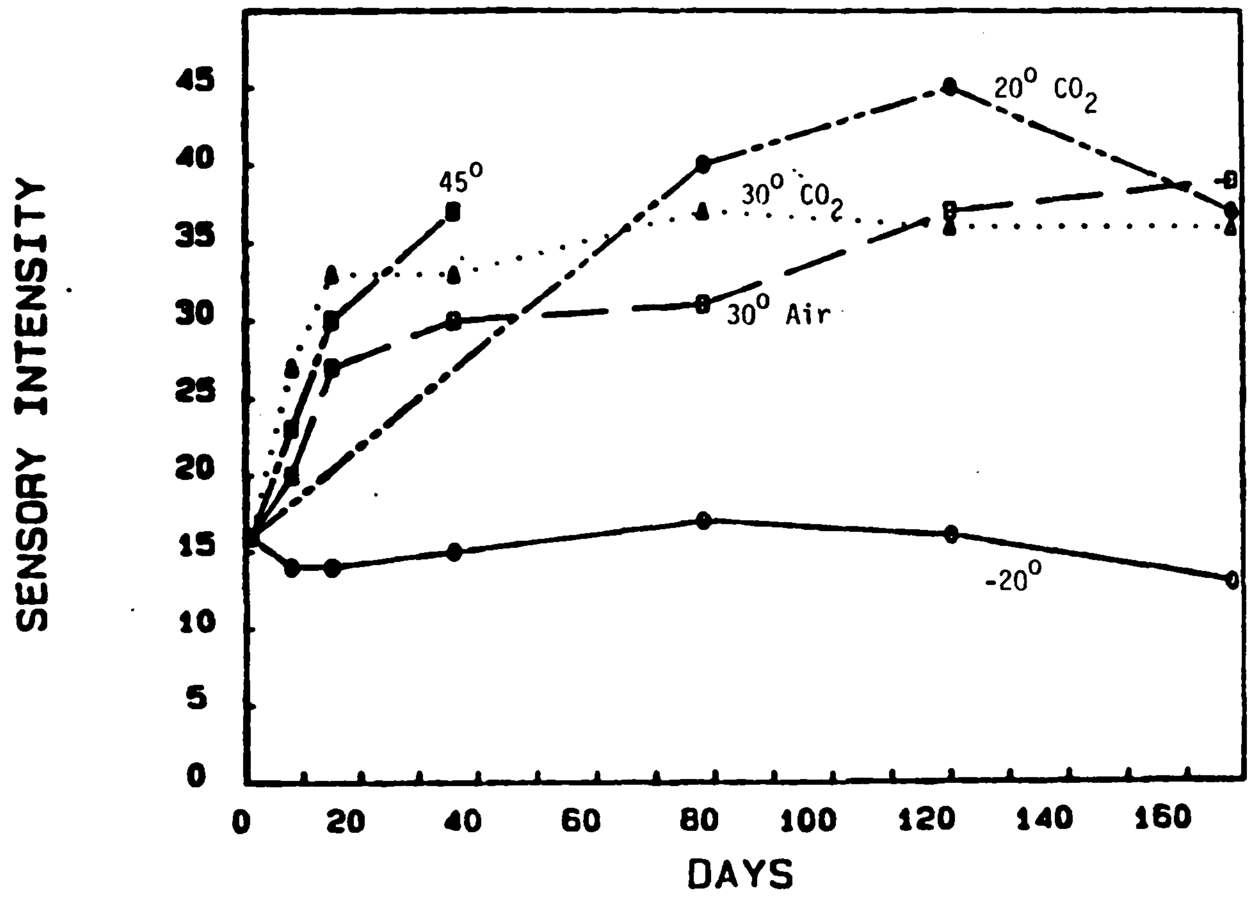

Figure 56. Effect of storage conditions on firmness of humectant bread (6-1.5-2).

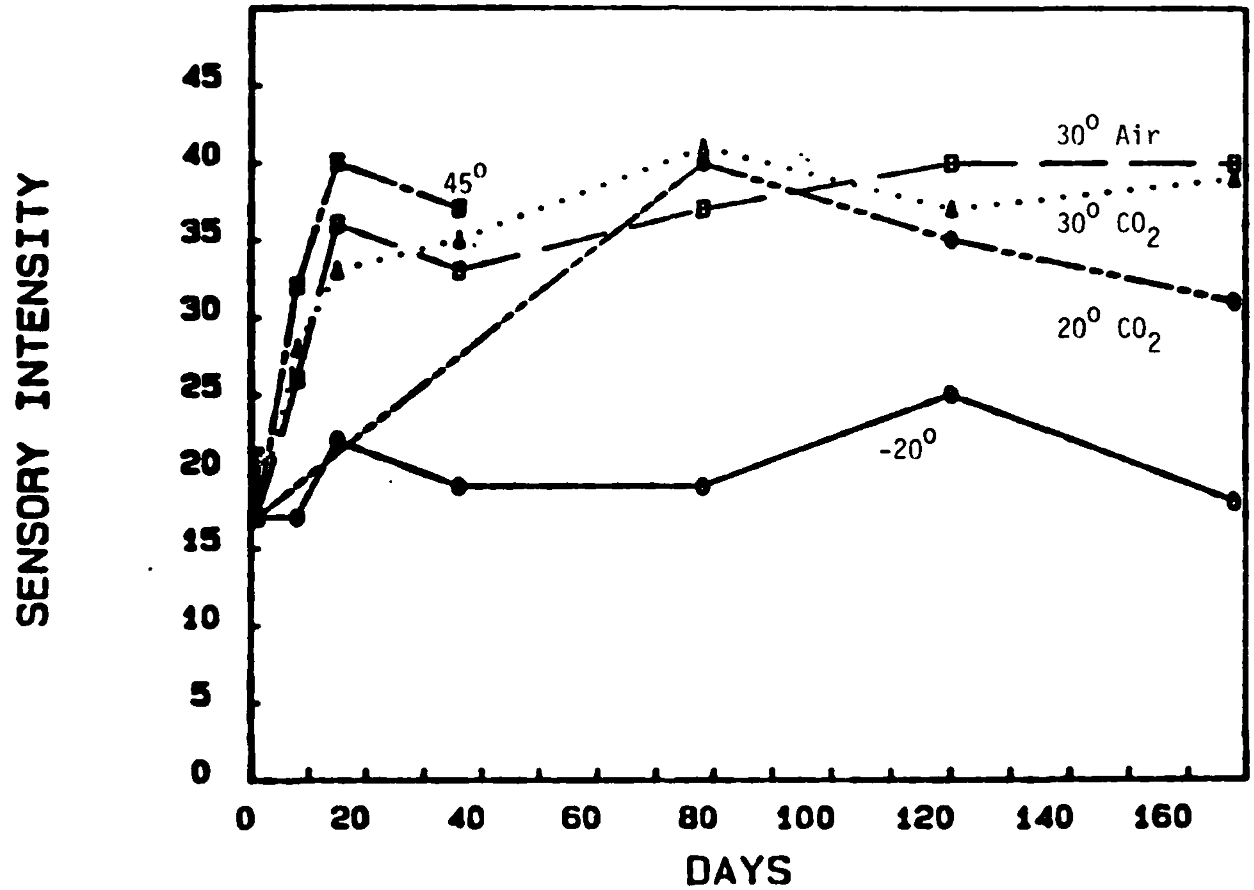

Figure 57. Effect of storage conditions on springiness of humectant bread (6-1.5-2).

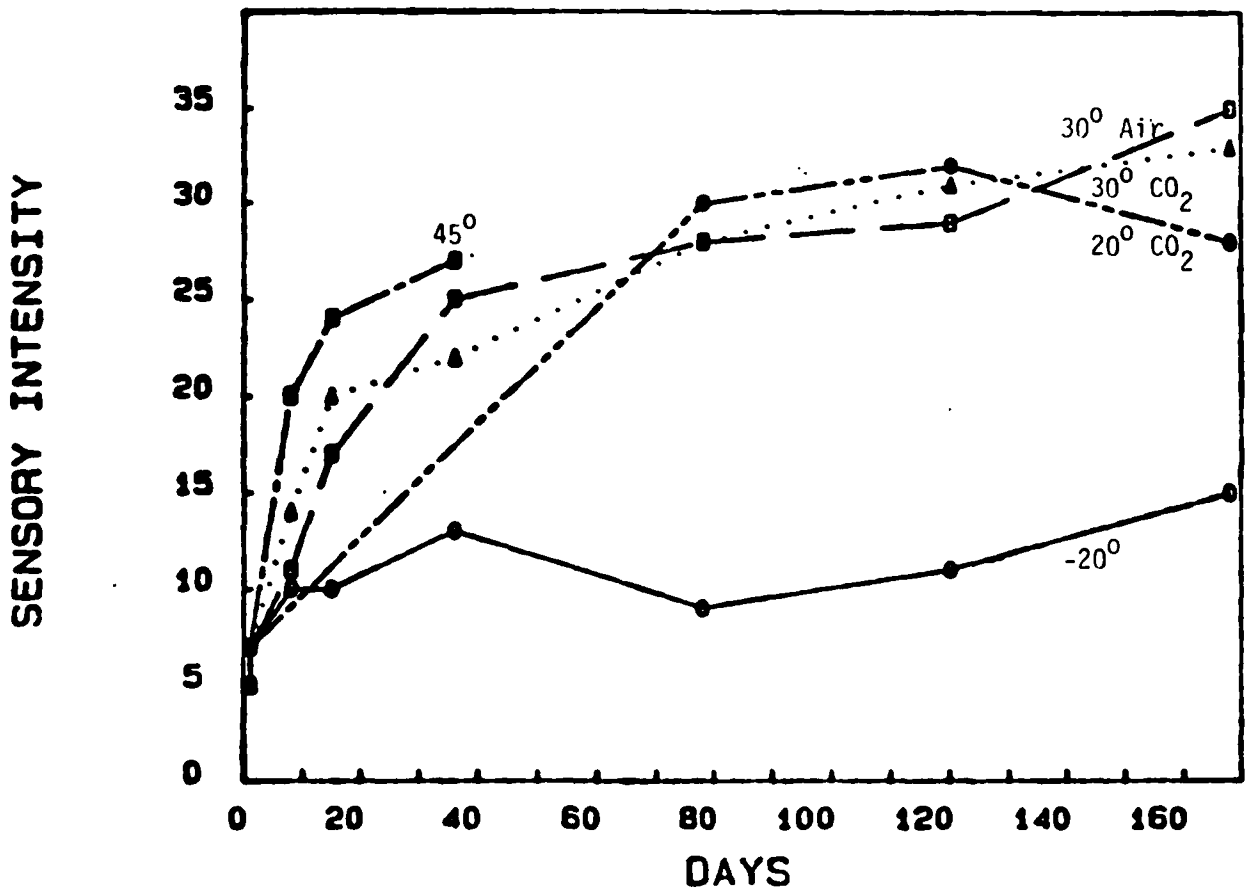

Figure 58. Effect of storage conditions on crumbliness of humectant bread (6-1.5-2).

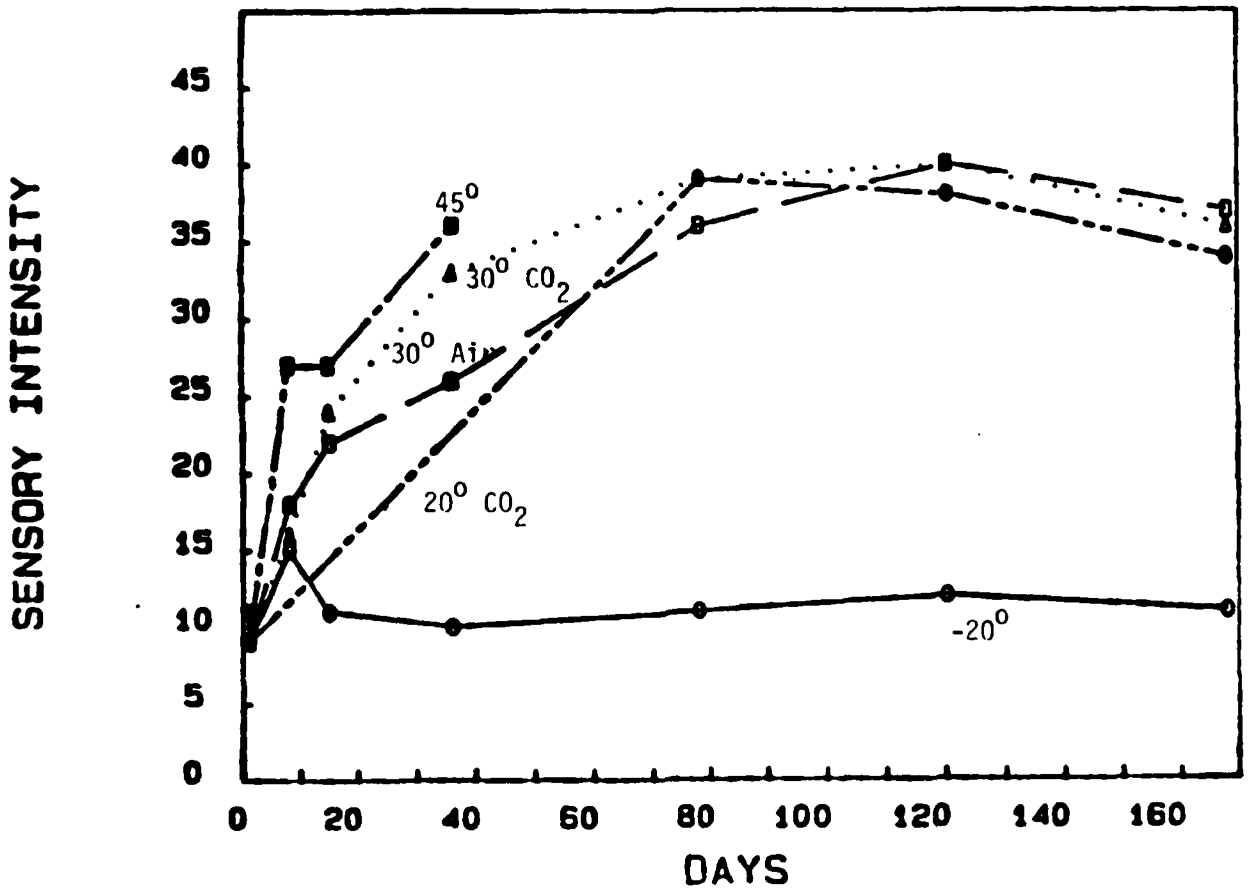

Figure 59. Effect of storage conditions on dryness (mouthfeel) of humectant bread (6-1.5-2).

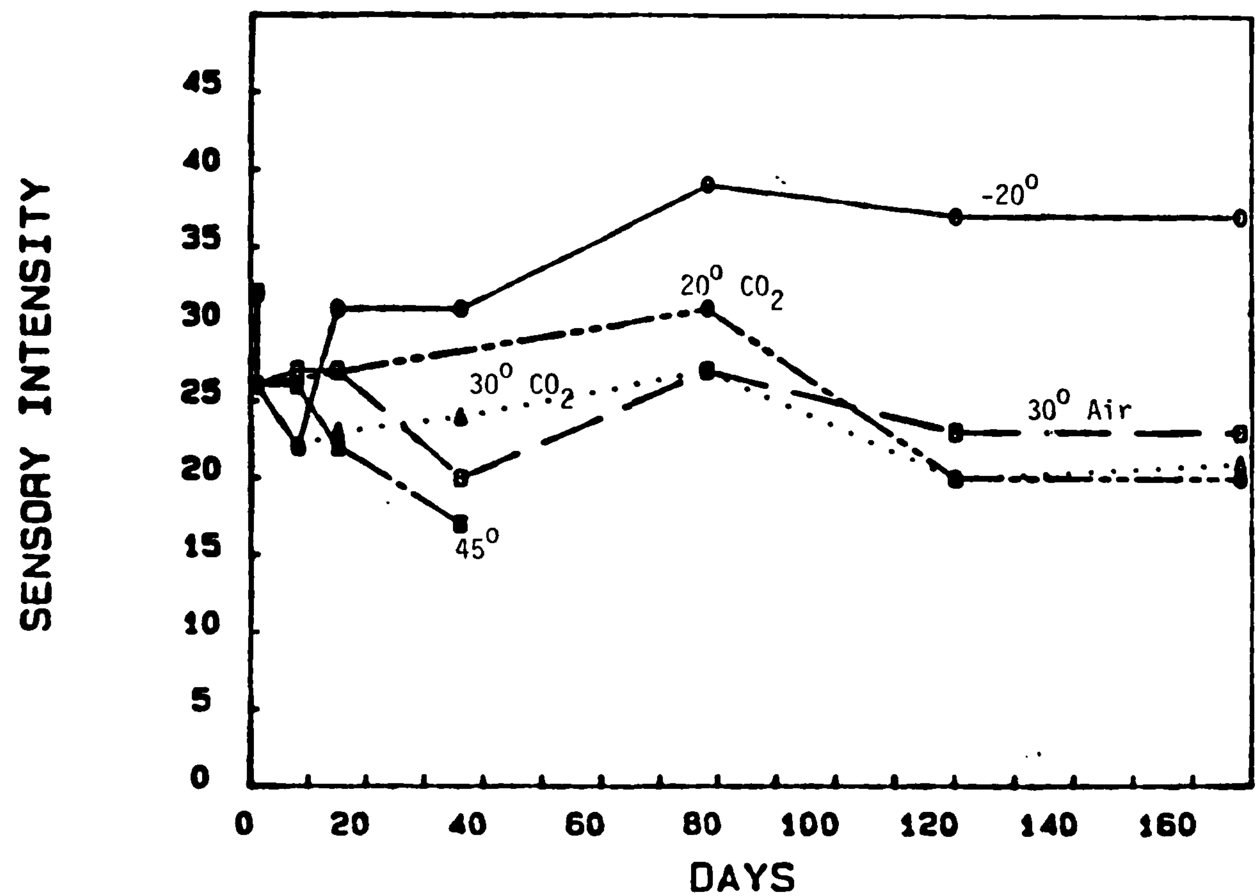

Figure 60. Effect of storage conditions on gumminess of humectant bread (6-1.5-2).

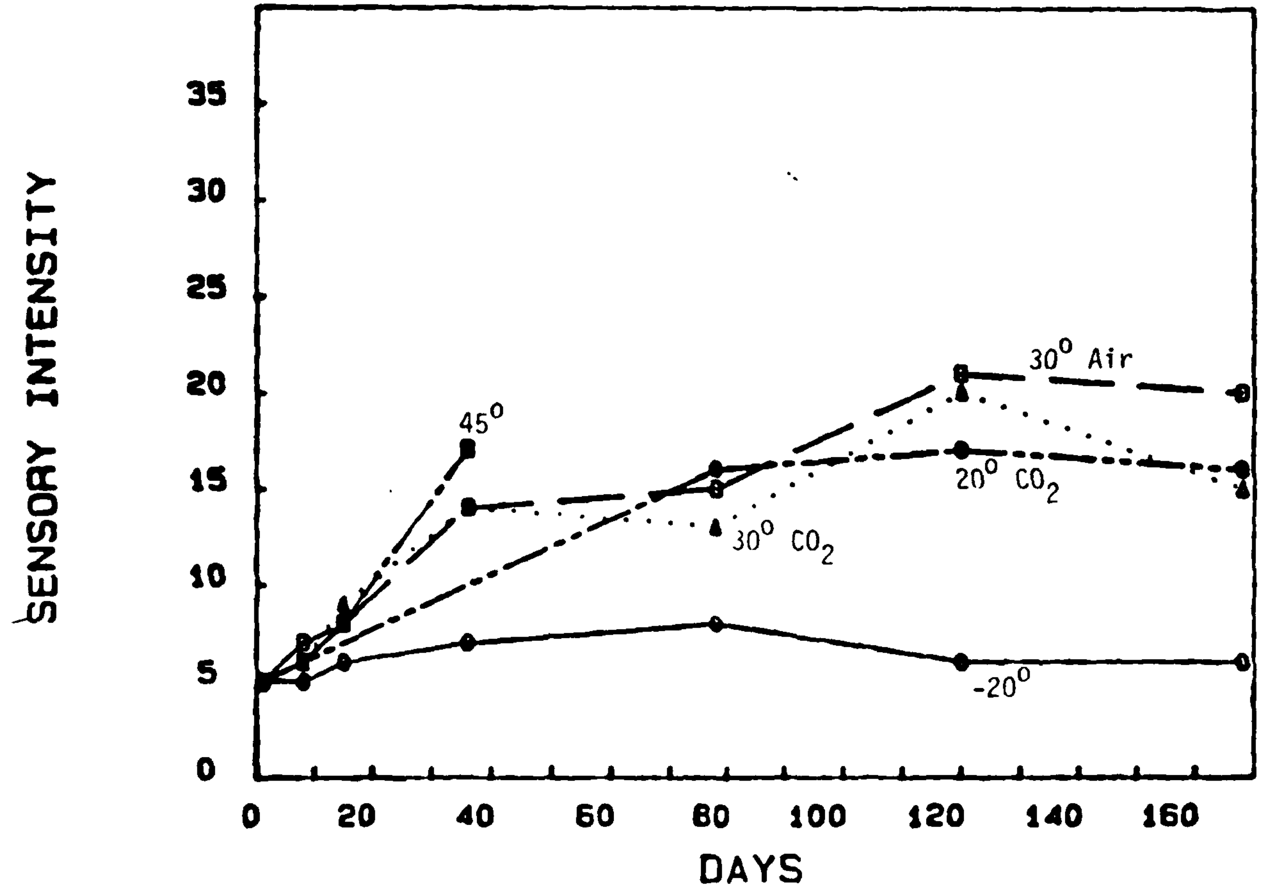

Figure 61. Effect of storage conditions on graininess of humectant bread (6-1.5-2).

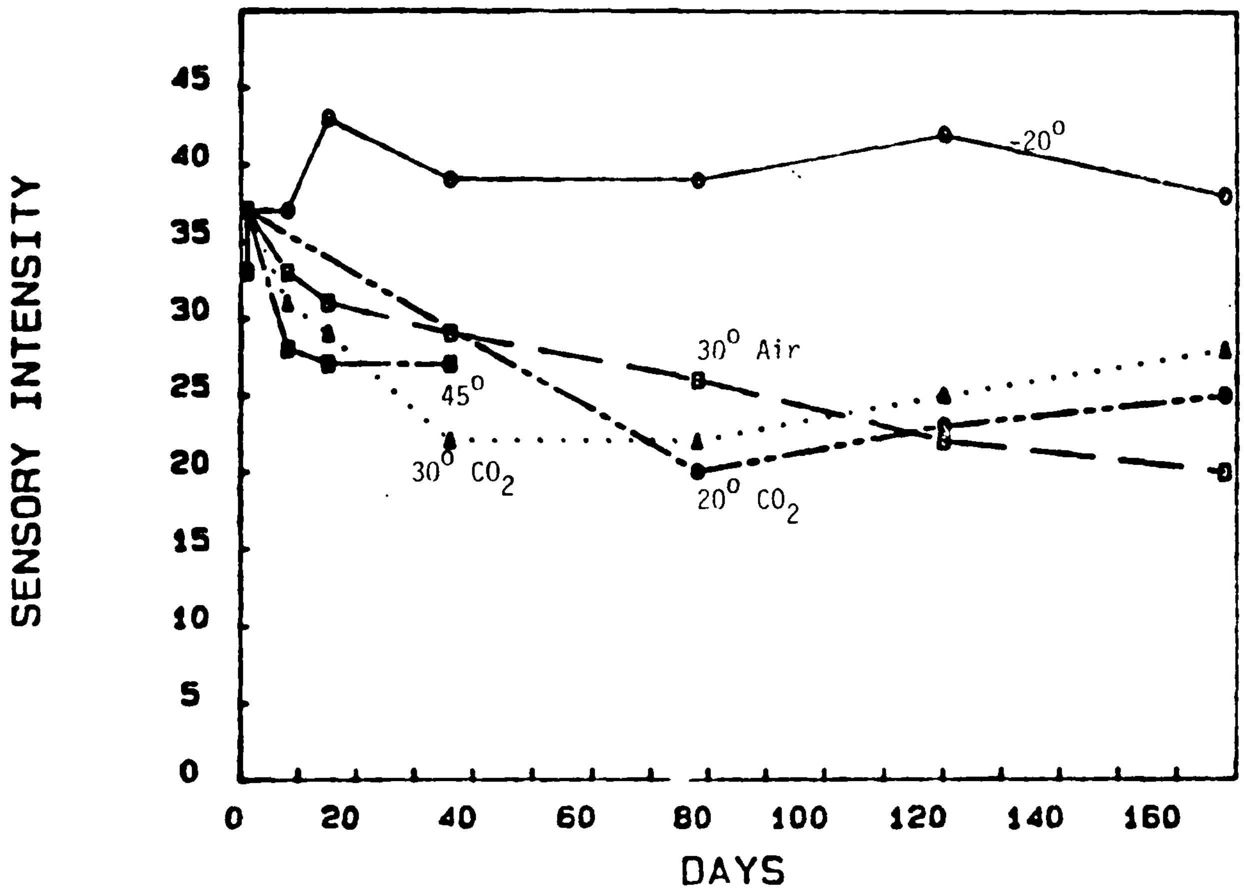

Figure 62. Effect of storage conditions on rate of hydration of humectant bread (6-1.5-2).

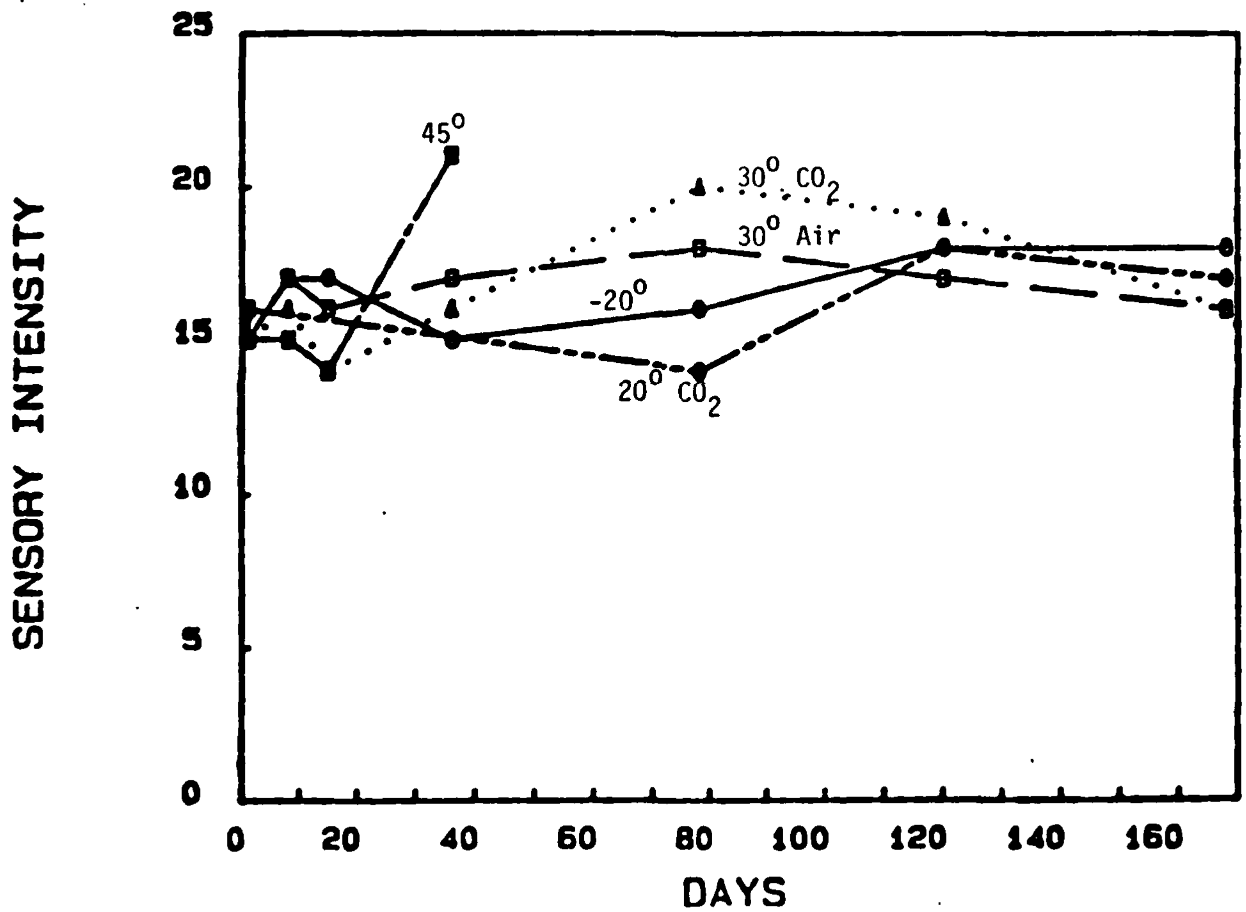

Figure 63. Effect of storage conditions on sweet flavor of crumb in humectant bread (6-1.5-2).

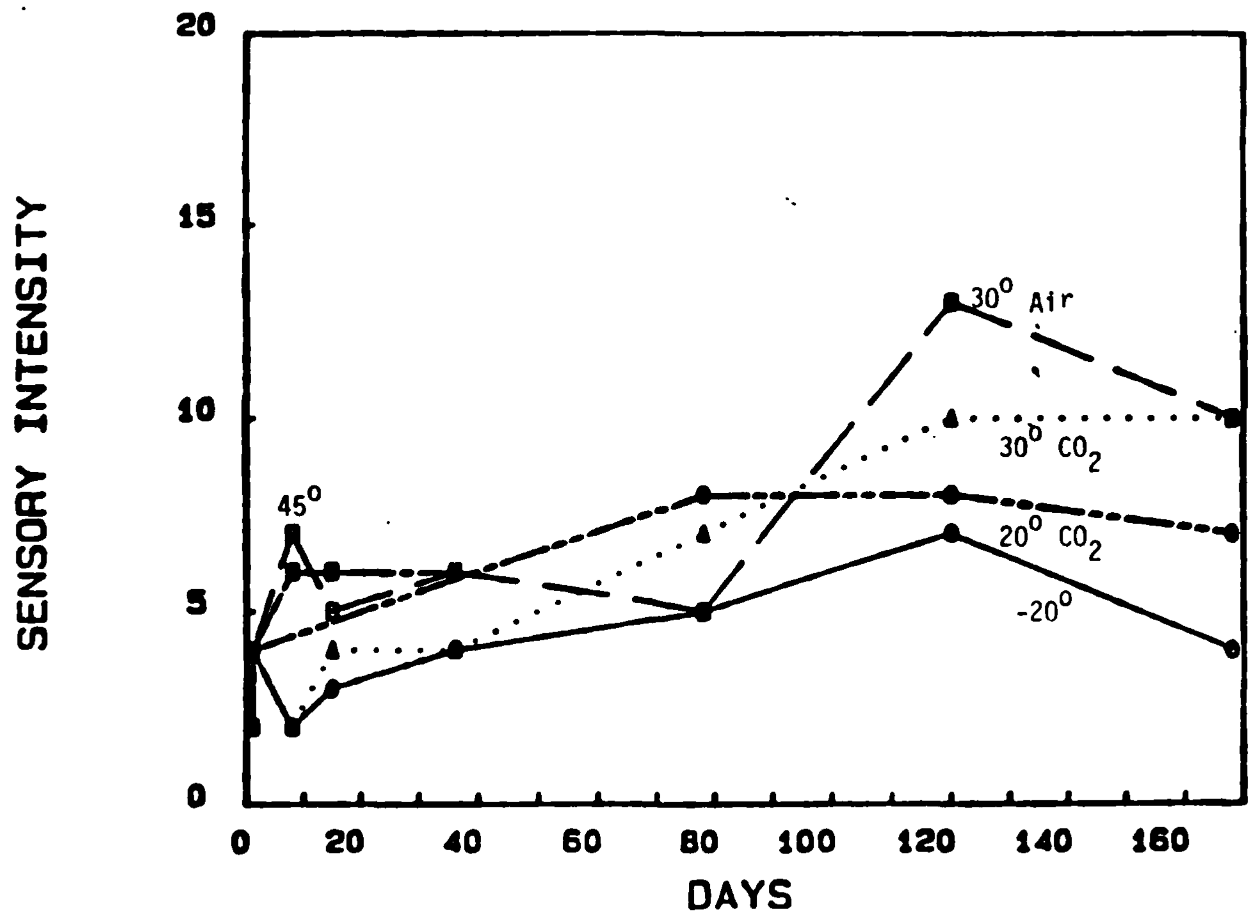

Figure 64. Effect of storage conditions on sour flavor of crumb in humectant bread (6-1.5-2).

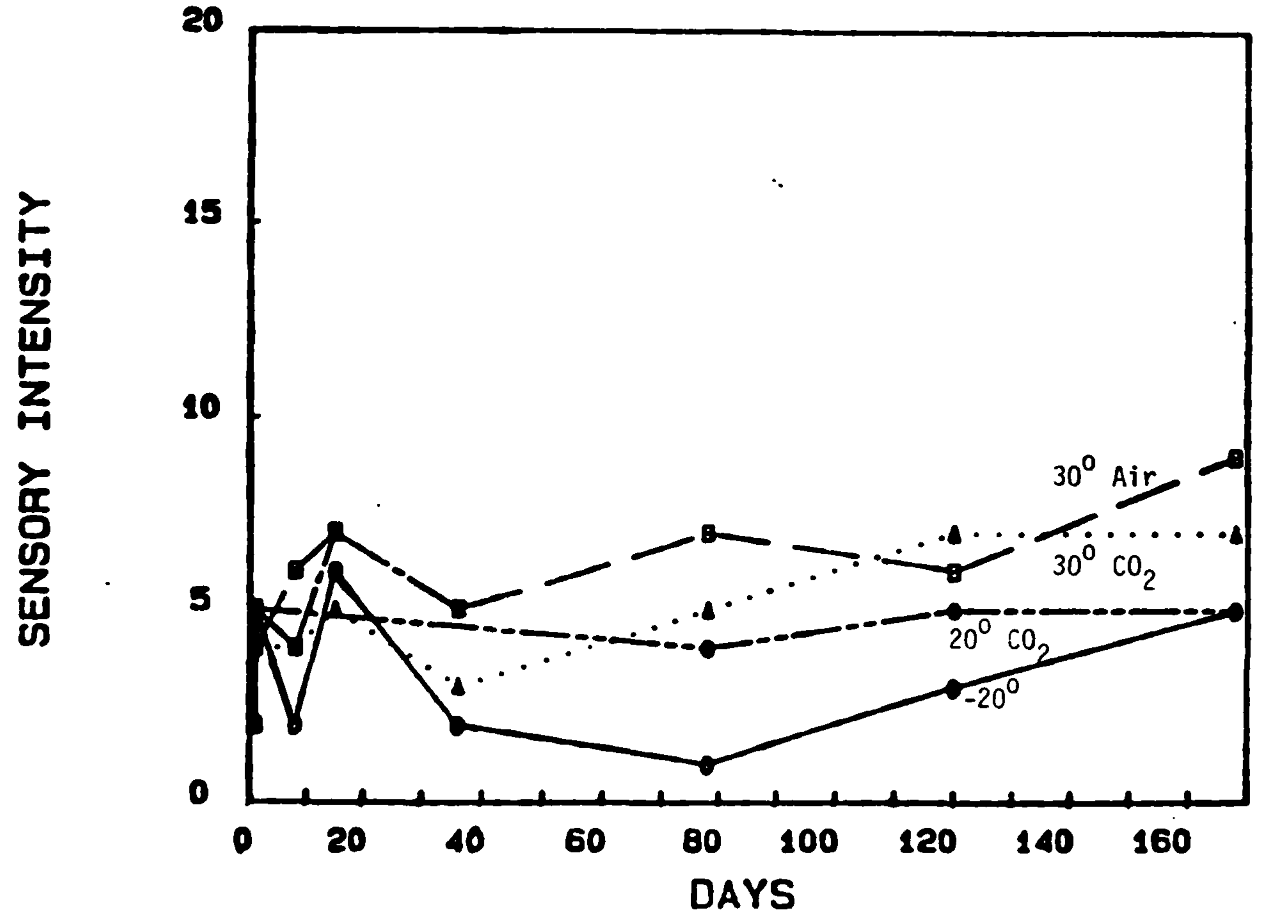

Figure 65. Effect of storage conditions on bitter flavor of crumb in humectant bread (6-1.5-2).

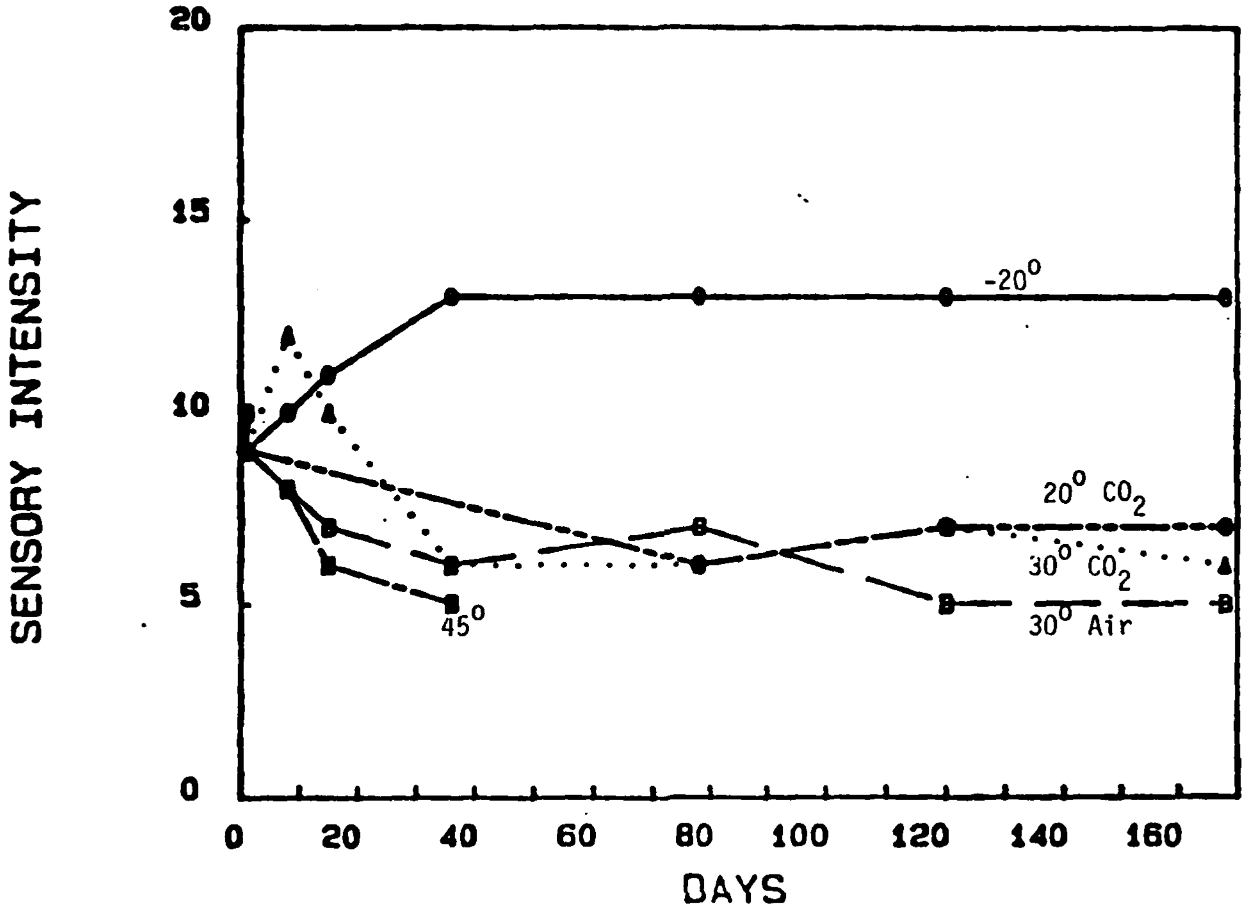

Figure 66. Effect of storage conditions on yeasty flavor of crumb in humectant bread (6-1.5-2).

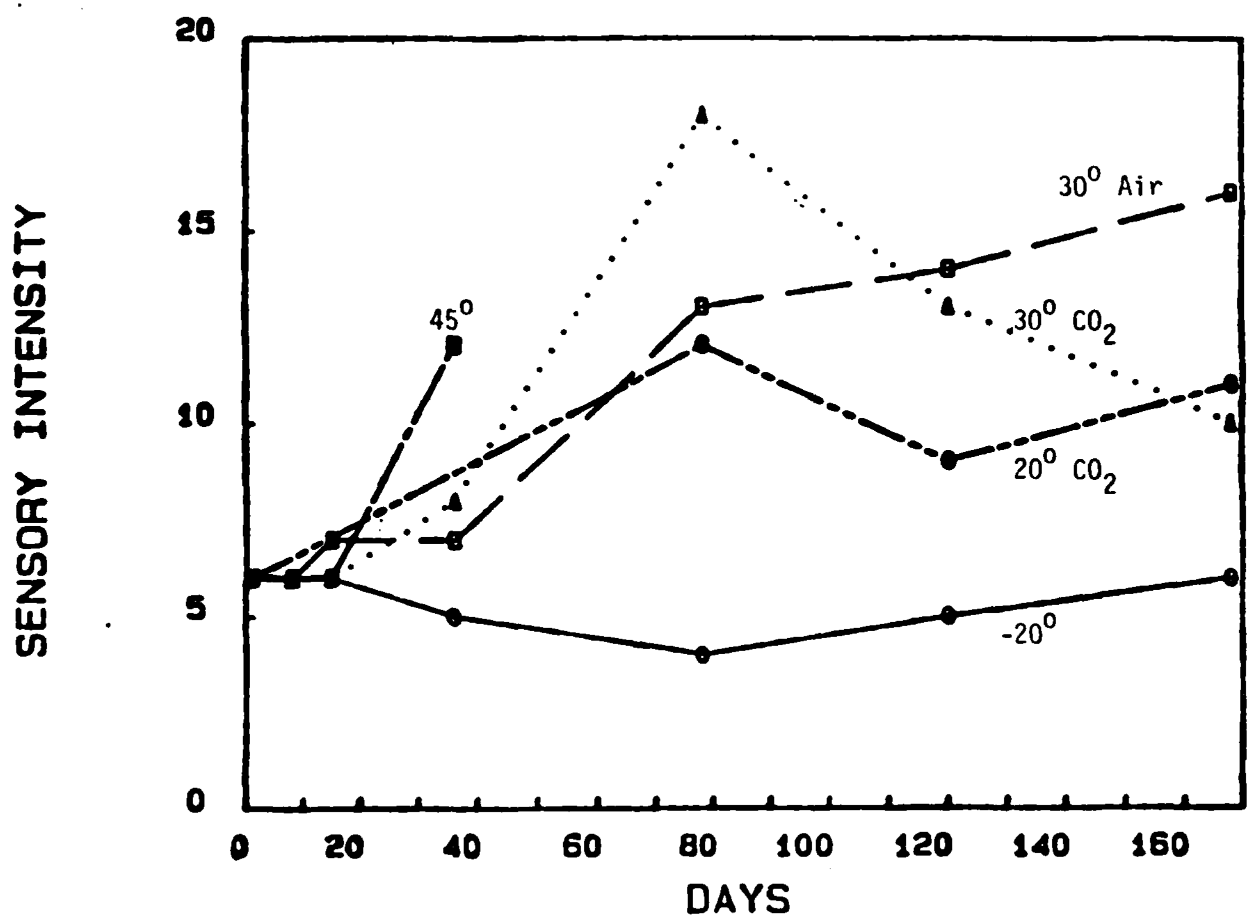

Figure 67. Effect of storage conditions on caramel flavor of crumb in humectant bread (6-1.5-2).

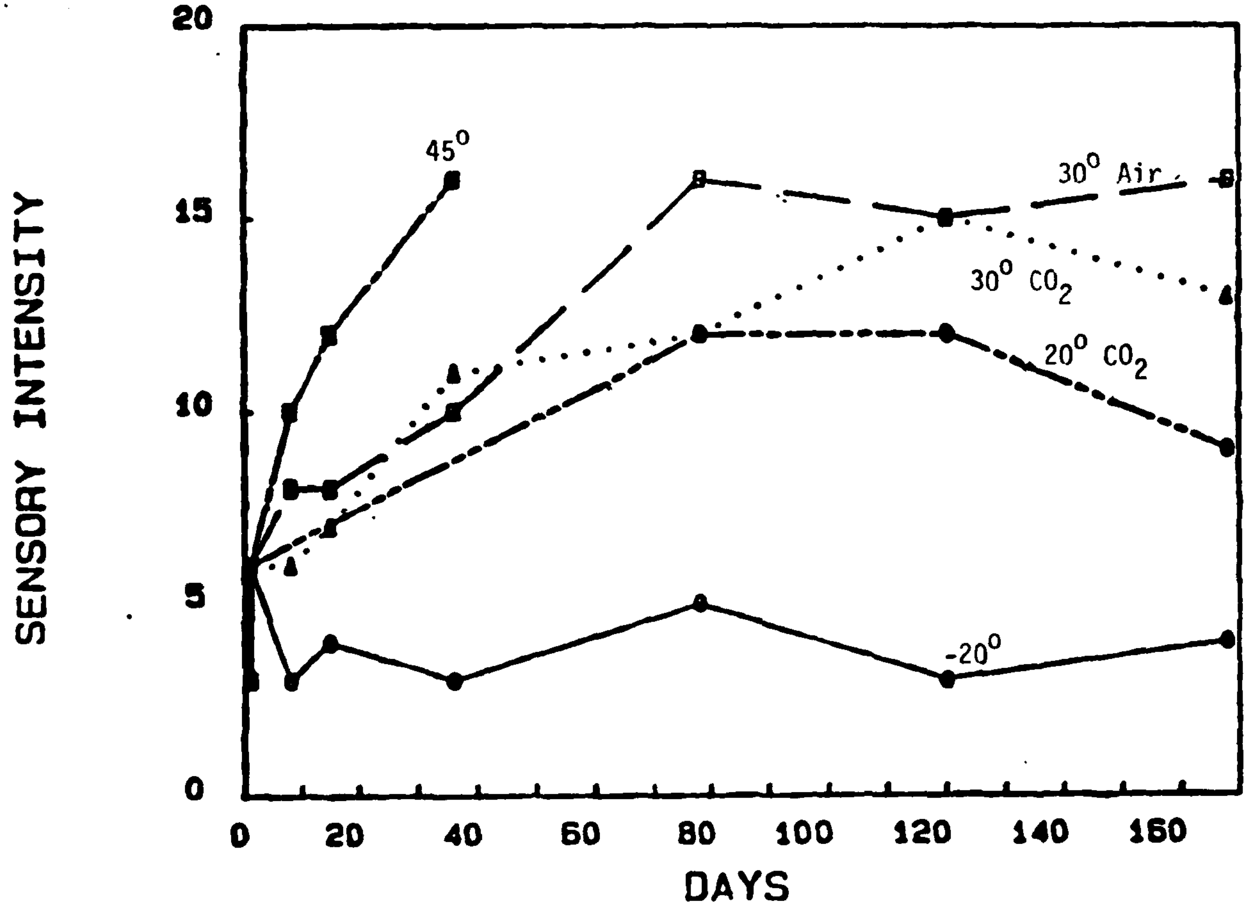

Figure 68. Effect of storage conditions on rancid flavor of crumb in humectant bread (6-1.5-2).

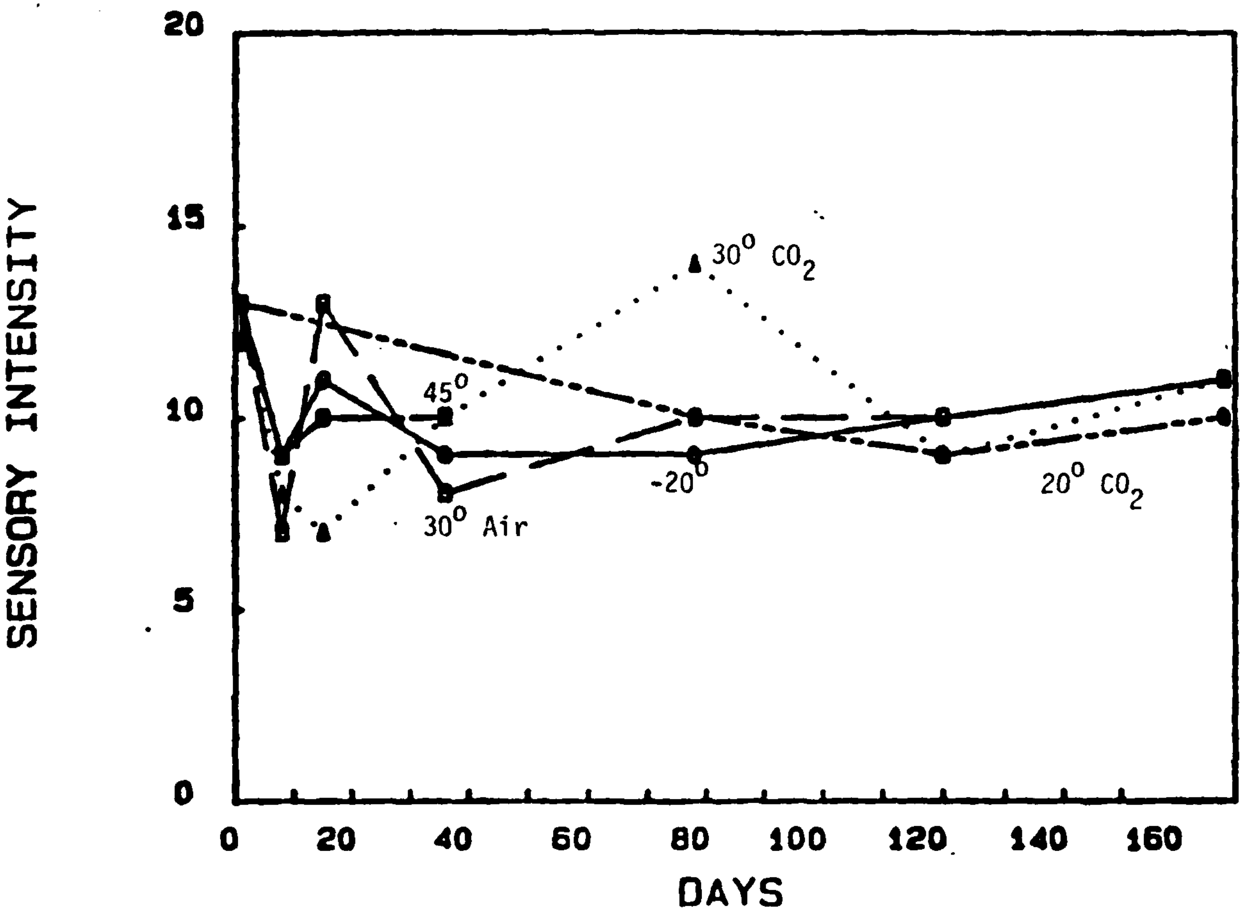

Figure 69. Effect of storage conditions on burnt flavor of crust of humectant bread (6-1.5-2).

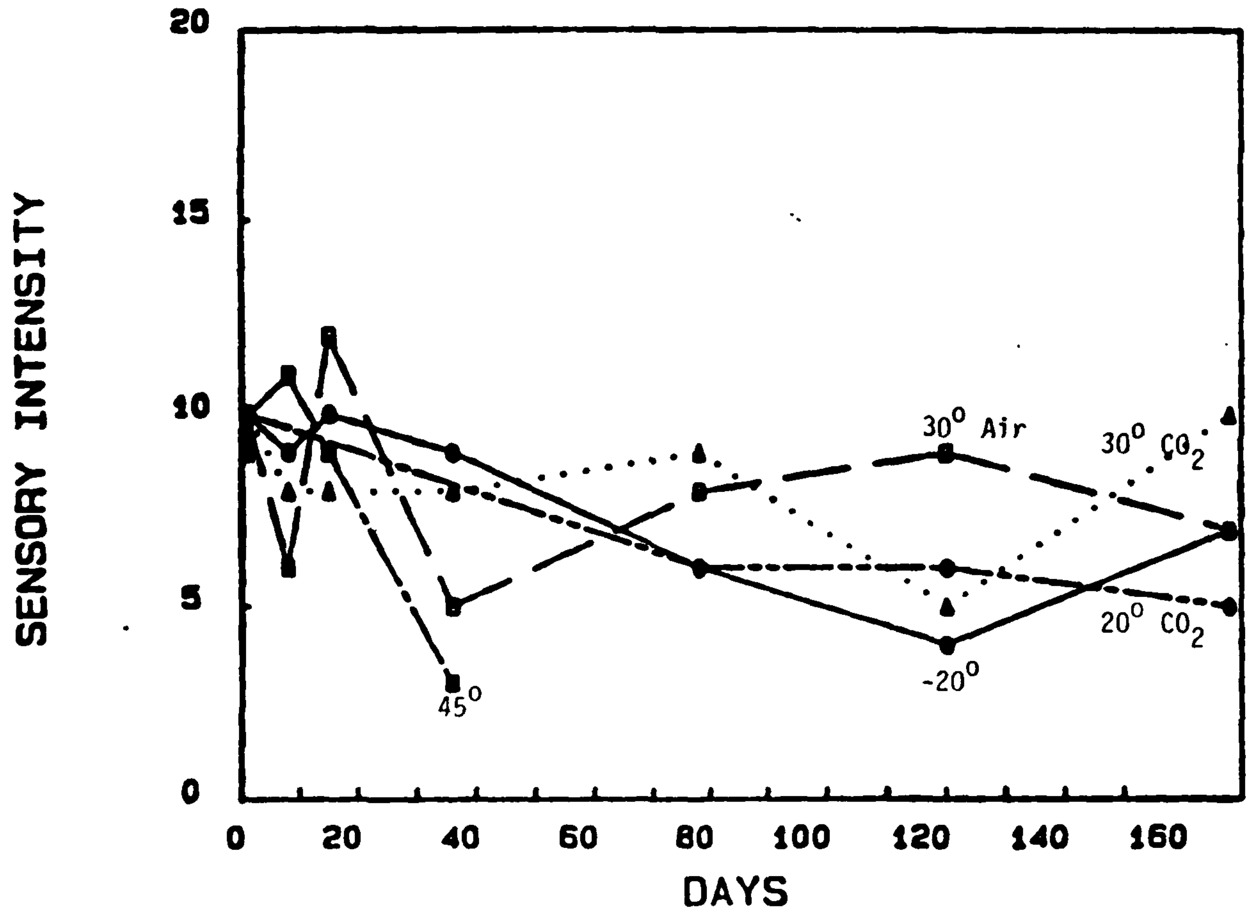

Figure 70. Effect of storage conditions on bitter flavor of crust of humectant bread (6-1.5-2).

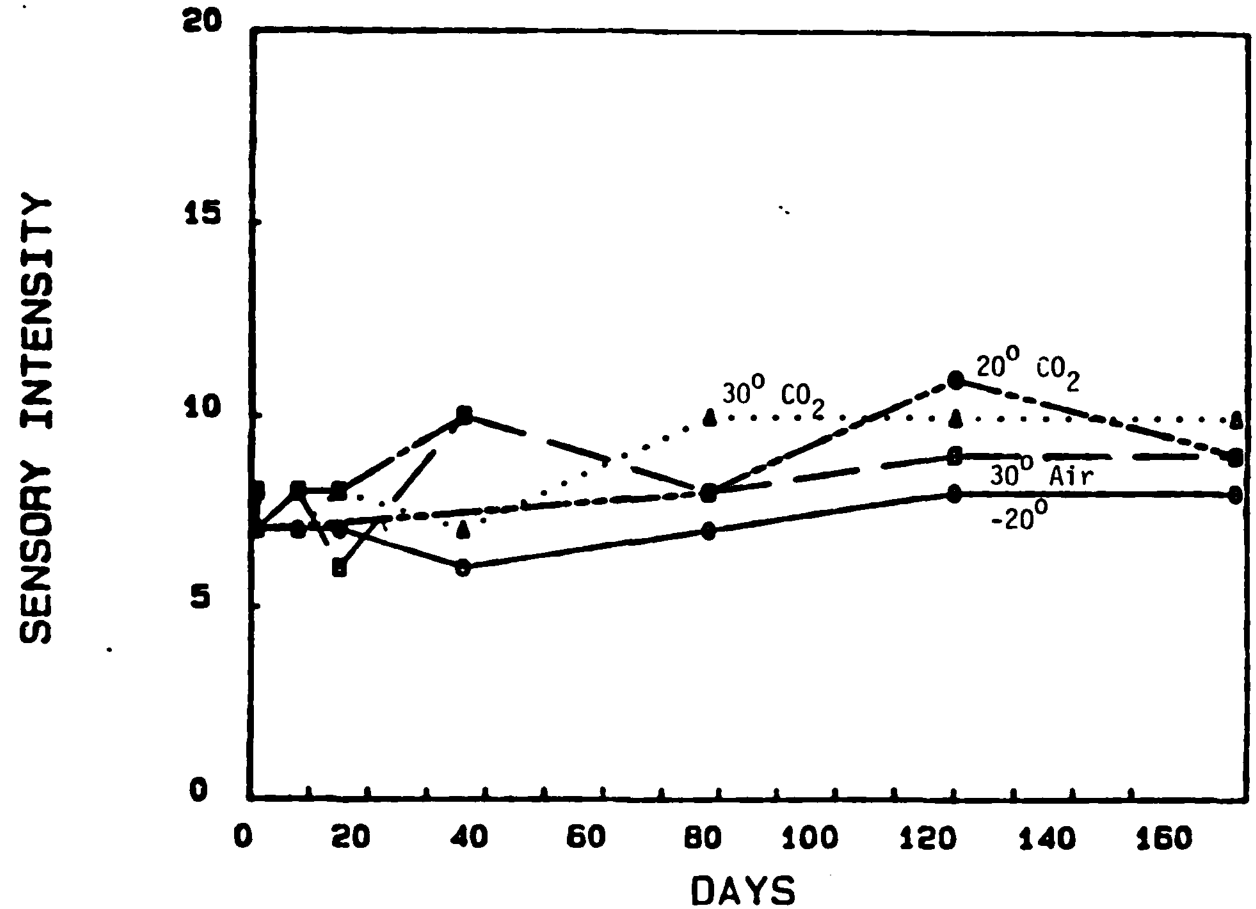

Figure 71. The effect of storage conditions on sweet flavor of crust of humectant bread (6-1.5-2).

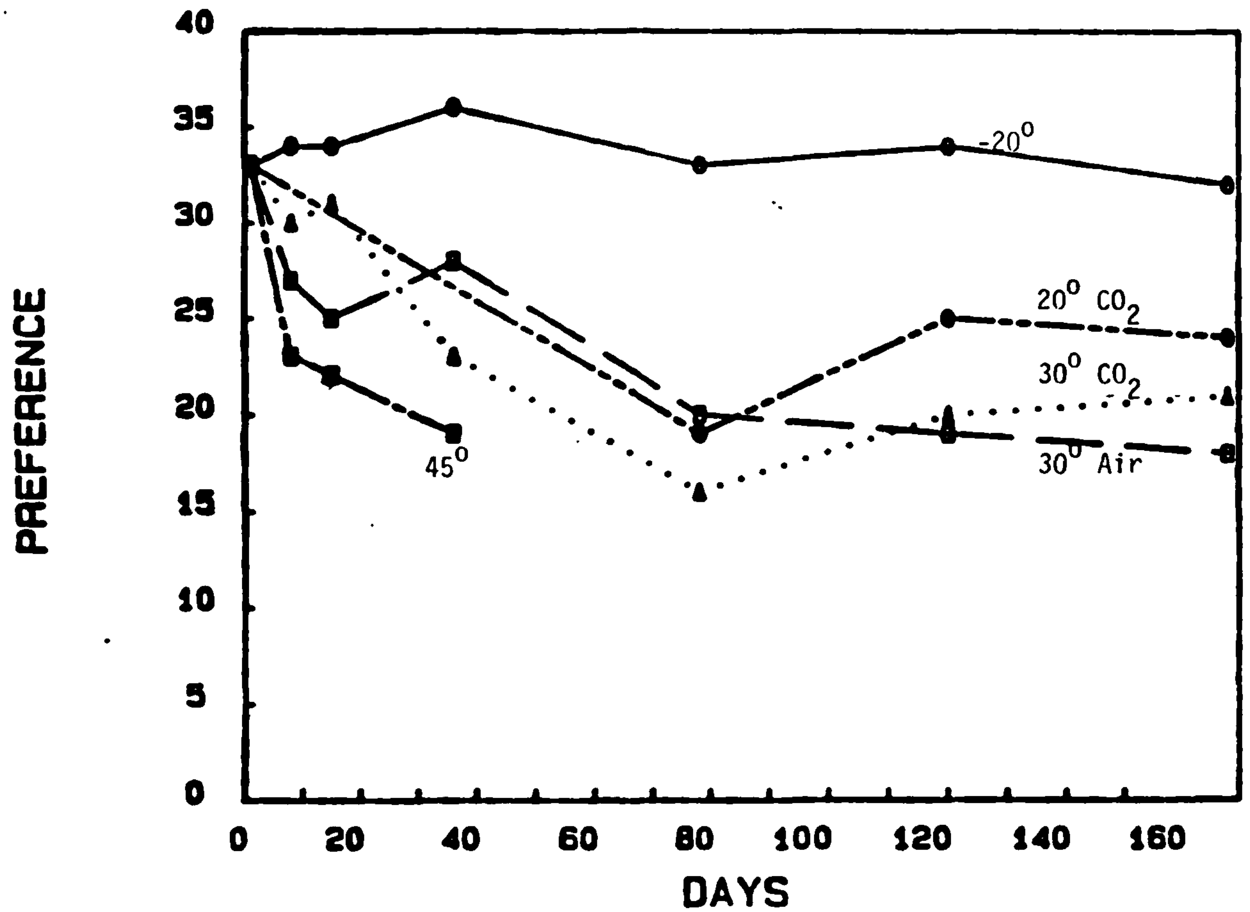

Figure 72. The effect of storage conditions on overall preference of humectant bread (6-1.5-2).

Appendix A.

PREFERENCE TEST OF BREAD

Name: ______________________ Date: April 19, 1984

Please evaluate the bread samples for overall acceptability and indicate your preference by encircling the appropriate number on the scale.

	Like Extremely	Like Very Much	Like Moderately	Like Slightly	Neither Like Nor Dislike	Dislike Slightly	Dislike Moderately	Dislike Very Much	Dislike Extremely
Code: 960	9	8	7	6	5	4	3	2	1
Code: 763	9	8	7	6	5	4	3	2	1
Code: 168	9	8	7	6	5	4	3	2	1
Code: 605	9	8	7	6	5	4	3	2	1
Code: 421	9	8	7	6	5	4	3	2	1

Appendix B

Name ______________________

Date ______________________

Please evaluate these samples on a scale of 0 to 5 for crumb texture, off-flavor, and overall preference.

Crumb texture:	0 = smooth and moist	
	5 = coarse and dry	
Off-flavor:	0 = imperceptible	
	5 = pronounced	
Overall preference:	0 = dislike very much	
	1 = dislike moderately	
	2 = dislike slightly	
	3 = like slightly	
	4 = like moderately	
	5 = like very much	

Code	Crumb texture	Off-flavor	Overall preference
___	__________	________	________
___	__________	________	________
___	__________	________	________
___	__________	________	________

Comments:

Appendix C

SAMPLE__________ BREAD EVALUATION NAME______________________

DATE______________________

Directions: Indicate the intensity of each characteristic by placing a vertical line through the horizontal scale.

APPEARANCE

crust browness	light		dark
crumb color	white	lt. yellow	brown

AROMA

overall intensity	slight	very
yeasty		
acrid		
sour		
rancid		
other (specify)		

TEXTURE

dryness	slight	very
firmness		
springiness		
crumbliness		

Appendix C

SAMPLE________ NAME____________________

MOUTHFEEL

dryness	slight	very
gumminess		
graininess		
rate of hydration	slow	fast

FLAVOR OF CRUMB

sweet	slight	very
sour		
bitter		
yeasty		
caramel		
cardboard/ rancid		
other (specify)		

FLAVOR OF CRUST

burnt	slight	very
bitter		
sweet		

OVERALL PREFERENCE

	disliked extremely	neither dislike or like	like extremely

Appendix C

BREAD EVALUATION

DEFINITIONS

TEXTURE

Dryness - lack of surface moisture

Firmness - resistance to squeezing between thumb and forefinger

Springiness - how fast sample springs back to original shape after deformation

Toughness - how much sample resists being torn apart

Crumbliness - how sample falls apart when apply shearing force

MOUTHFEEL

Dryness - Lack of surface moisture on tongue, abrasive

Toughness - resistance to bite

Stickiness - how much sample sticks to teeth and palate (adhesive like peanut butter)

Gumminess - how much the sample sticks to itself (like chewing gum)

Rate of hydration - how easily the sample took up moisture while chewing

Graininess - feel particles on the palate while masticating

Appendix D

SCORECARD FOR PREFERENCE TEST

NAME________________________

DATE________________________

CODE________________________

SAMPLE #__________

Directions: Please taste the sample and rate how much you like it by placing a vertical line on the scale below.

Dislike Extremely	Neither Dislike or Like	Like Extremely

Comments:

NAME________________________

DATE________________________

CODE________________________

SAMPLE #__________

Directions: Please taste the sample and rate how much you like it by placing a vertical line on the scale below.

Dislike Extremely	Neither Dislike or Like	Like Extremely

Comments:

Appendix E

----------VARIABLE=WATER ACT TYPES=CRUMB----------

OBS	AIR	REP	FORMULA	VALUE	DAY
261	30_CO2	1	6:15:2	0.949	1
262	30_CO2	2	6:15:2	0.949	1
263	30_CO2	1	6:15:2	0.941	8
264	30_CO2	2	6:15:2	0.941	8
265	30_CO2	1	6:15:2	0.933	15
266	30_CO2	2	6:15:2	0.934	15
267	30_CO2	1	6:15:2	0.927	22
268	30_CO2	2	6:15:2	0.929	22
269	30_CO2	1	6:15:2	0.924	43
270	30_CO2	2	6:15:2	0.920	43
271	30_CO2	1	6:15:2	0.934	65
272	30_CO2	2	6:15:2	0.932	65
273	30_CO2	1	6:15:2	0.934	85
274	30_CO2	2	6:15:2	0.930	85
275	30_CO2	1	6:15:2	0.932	113
276	30_CO2	2	6:15:2	0.933	113
277	30_CO2	1	6:15:2	0.933	141
278	30_CO2	2	6:15:2	0.929	141
279	30_CO2	1	6:15:2	0.920	169
280	30_CO2	2	6:15:2	0.919	169
281	30_CO2	1	7:15:2	0.949	1
282	30_CO2	2	7:15:2	0.949	1
283	30_CO2	1	7:15:2	0.935	8
284	30_CO2	2	7:15:2	0.940	8
285	30_CO2	1	7:15:2	0.927	15
286	30_CO2	2	7:15:2	0.928	15
287	30_CO2	1	7:15:2	0.924	22
288	30_CO2	2	7:15:2	0.918	22
289	30_CO2	1	7:15:2	0.913	43
290	30_CO2	2	7:15:2	0.924	43
291	30_CO2	1	7:15:2	0.921	65
292	30_CO2	2	7:15:2	0.926	65
293	30_CO2	1	7:15:2	0.930	85
294	30_CO2	2	7:15:2	0.928	85
295	30_CO2	1	7:15:2	0.927	113
296	30_CO2	2	7:15:2	0.928	113
297	30_CO2	1	7:15:2	0.924	141
298	30_CO2	2	7:15:2	0.926	141
299	30_CO2	1	7:15:2	0.914	169
300	30_CO2	2	7:15:2	0.914	169

Appendix E

-----------VARIABLE=WATER ACT (TCS+CRUST)-----------

OBS	AIR	REP	FORMULA	VALUE	DAY
301	30_CO2	1	6:15:2	0.925	15
302	30_CO2	2	6:15:2	0.917	15
303	30_CO2	1	6:15:2	0.919	22
304	30_CO2	2	6:15:2	0.921	22
305	30_CO2	1	6:15:2	0.909	43
306	30_CO2	2	6:15:2	0.910	43
307	30_CO2	1	6:15:2	0.923	65
308	30_CO2	2	6:15:2	0.932	65
309	30_CO2	1	6:15:2	0.932	85
310	30_CO2	2	6:15:2	0.927	85
311	30_CO2	1	6:15:2	0.916	113
312	30_CO2	2	6:15:2	0.914	113
313	30_CO2	1	6:15:2	0.928	141
314	30_CO2	2	6:15:2	0.930	141
315	30_CO2	1	6:15:2	0.915	169
316	30_CO2	2	6:15:2	0.918	169
317	30_CO2	1	7:15:2	0.916	15
318	30_CO2	2	7:15:2	0.914	15
319	30_CO2	1	7:15:2	0.913	22
320	30_CO2	2	7:15:2	0.918	22
321	30_CO2	1	7:15:2	0.901	43
322	30_CO2	2	7:15:2	0.911	43
323	30_CO2	1	7:15:2	0.923	65
324	30_CO2	2	7:15:2	0.917	65
325	30_CO2	1	7:15:2	0.925	85
326	30_CO2	2	7:15:2	0.922	85
327	30_CO2	1	7:15:2	0.905	113
328	30_CO2	2	7:15:2	0.908	113
329	30_CO2	1	7:15:2	0.919	141
330	30_CO2	2	7:15:2	0.925	141
331	30_CO2	1	7:15:2	0.911	169
332	30_CO2	2	7:15:2	0.911	169

----------VARIABLE=PH TYPES=CRUST----------

OBS	AIR	REP	FORMULA	VALUE	DAY
197	30_CO2	1	6:15:2	5.00	1
198	30_CO2	2	6:15:2	5.03	1
199	45_CO2	1	6:15:2	5.00	1
200	45_CO2	2	6:15:2	5.02	1
201	30_CO2	1	6:15:2	5.06	8
202	30_CO2	2	6:15:2	5.10	8
203	45_CO2	1	6:15:2	5.03	8
204	45_CO2	2	6:15:2	4.97	8
205	30_CO2	1	6:15:2	5.17	15
206	30_CO2	2	6:15:2	5.14	15
207	45_CO2	1	6:15:2	5.11	15
208	45_CO2	2	6:15:2	5.15	15
209	30_CO2	1	6:15:2	5.08	22
210	30_CO2	2	6:15:2	5.09	22
211	45_CO2	1	6:15:2	5.04	22
212	45_CO2	2	6:15:2	5.05	22
213	30_CO2	1	6:15:2	5.09	43
214	30_CO2	2	6:15:2	5.07	43
215	45_CO2	1	6:15:2	4.88	43
216	45_CO2	2	6:15:2	4.95	43
217	30_CO2	1	6:15:2	5.11	65
218	30_CO2	2	6:15:2	5.05	65
219	45_CO2	1	6:15:2	4.76	65
220	45_CO2	2	6:15:2	4.77	65
221	30_CO2	1	6:15:2	4.84	85
222	30_CO2	2	6:15:2	4.94	85
223	30_CO2	1	6:15:2	4.92	113
224	30_CO2	2	6:15:2	4.97	113
225	30_CO2	1	6:15:2	4.95	141
226	30_CO2	2	6:15:2	4.96	141
227	30_CO2	1	6:15:2	4.82	169
228	30_CO2	2	6:15:2	4.83	169
229	30_CO2	1	7:15:2	4.98	1
230	30_CO2	2	7:15:2	4.98	1
231	45_CO2	1	7:15:2	4.98	1
232	45_CO2	2	7:15:2	4.98	1
233	30_CO2	1	7:15:2	5.04	8
234	30_CO2	2	7:15:2	4.96	8
235	45_CO2	1	7:15:2	4.96	8
236	45_CO2	2	7:15:2	4.97	8
237	30_CO2	1	7:15:2	5.09	15
238	30_CO2	2	7:15:2	5.09	15
239	45_CO2	1	7:15:2	4.99	15
240	45_CO2	2	7:15:2	5.06	15
241	30_CO2	1	7:15:2	4.99	22
242	30_CO2	2	7:15:2	5.00	22
243	45_CO2	1	7:15:2	4.92	22
244	45_CO2	2	7:15:2	4.94	22
245	30_CO2	1	7:15:2	5.05	43
246	30_CO2	2	7:15:2	5.01	43
247	45_CO2	1	7:15:2	4.81	43
248	45_CO2	2	7:15:2	4.86	43
249	30_CO2	1	7:15:2	4.95	65

Appendix F

-------------------VARIABLE=PH TYPES=CRUST-------------------

OBS	AIR	REP	FORMULA	VALUE	DAY
250	30_CO2	2	7:15:2	4.96	65
251	45_CO2	1	7:15:2	4.71	65
252	45_CO2	2	7:15:2	4.77	65
253	30_CO2	1	7:15:2	4.71	85
254	30_CO2	2	7:15:2	4.87	85
255	30_CO2	1	7:15:2	4.71	113
256	30_CO2	2	7:15:2	4.82	113
257	30_CO2	1	7:15:2	4.78	141
258	30_CO2	2	7:15:2	4.83	141
259	30_CO2	1	7:15:2	4.76	169
260	30_CO2	2	7:15:2	4.78	169

Appendix F

VARIABLE=FUDOH TYPES=CRUST

OBS	AIR	REP	FORMULA	VALUE	DAY
1	30_AIR	1	6:15:2	304.0	1
2	30_AIR	2	6:15:2	335.0	1
3	30_CO2	1	6:15:2	462.0	8
4	30_CO2	2	6:15:2	596.0	8
5	30_AIR	1	6:15:2	443.0	8
6	30_AIR	2	6:15:2	425.0	8
7	45_CO2	1	6:15:2	531.0	8
8	45_CO2	2	6:15:2	438.0	8
9	30_CO2	1	6:15:2	644.0	15
10	30_CO2	2	6:15:2	703.0	15
11	30_AIR	1	6:15:2	518.0	15
12	30_AIR	2	6:15:2	584.0	15
13	45_CO2	1	6:15:2	751.0	15
14	45_CO2	2	6:15:2	958.0	15
15	20_CO2	1	6:15:2	799.0	22
16	20_CO2	2	6:15:2	784.0	22
17	30_CO2	1	6:15:2	617.0	22
18	30_CO2	2	6:15:2	798.0	22
19	30_AIR	1	6:15:2	783.0	22
20	30_AIR	2	6:15:2	865.0	22
21	45_CO2	1	6:15:2	849.0	22
22	45_CO2	2	6:15:2	915.0	22
23	20_CO2	1	6:15:2	1093.0	43
24	20_CO2	2	6:15:2	1031.0	43
25	30_CO2	1	6:15:2	1077.0	43
26	30_CO2	2	6:15:2	1111.0	43
27	30_AIR	1	6:15:2	898.0	43
28	30_AIR	2	6:15:2	846.0	43
29	45_CO2	1	6:15:2	1069.0	43
30	45_CO2	2	6:15:2	1297.0	43
31	30_CO2	1	6:15:2	1083.0	65
32	30_CO2	2	6:15:2	992.0	65
33	30_AIR	1	6:15:2	1003.0	65
34	30_AIR	2	6:15:2	863.0	65
35	45_CO2	1	6:15:2	1263.0	65
36	45_CO2	2	6:15:2	1049.0	65
37	20_CO2	1	6:15:2	1140.0	85
38	20_CO2	2	6:15:2	1071.0	85
39	30_CO2	1	6:15:2	1108.0	85
40	30_CO2	2	6:15:2	993.0	85
41	30_AIR	1	6:15:2	974.0	85
42	30_AIR	2	6:15:2	1058.0	85
43	20_CO2	1	6:15:2	1015.0	113
44	20_CO2	2	6:15:2	1141.0	113
45	30_CO2	1	6:15:2	1035.0	113
46	30_CO2	2	6:15:2	1217.0	113
47	30_AIR	1	6:15:2	1177.0	113
48	30_AIR	2	6:15:2	1133.0	113
49	20_CO2	1	6:15:2	1003.0	141
50	20_CO2	2	6:15:2	1091.0	141
51	30_CO2	1	6:15:2	993.0	141
52	30_CO2	2	6:15:2	1047.0	141
53	30_AIR	1	6:15:2	862.0	141

---------------VARIABLE=FUDON TYPES=CRUST---------------

OBS	AIR	REP	FORMULA	VALUE	DAY
54	30_AIR	2	6:15:2	975.0	169
55	20_CO2	1	6:15:2	1145.0	169
56	20_CO2	2	6:15:2	1118.0	169
57	30_CO2	1	6:15:2	1047.0	169
58	30_CO2	2	6:15:2	1111.0	169
59	30_AIR	1	6:15:2	1008.0	169
60	30_AIR	2	6:15:2	1064.0	169
61	30_AIR	1	7:15:2	343.8	1
62	30_AIR	2	7:15:2	364.2	1
63	30_CO2	1	7:15:2	605.0	8
64	30_CO2	2	7:15:2	565.0	8
65	30_AIR	1	7:15:2	503.0	8
66	30_AIR	2	7:15:2	655.0	8
67	45_CO2	1	7:15:2	609.0	8
68	45_CO2	2	7:15:2	618.0	8
69	30_CO2	1	7:15:2	699.0	15
70	30_CO2	2	7:15:2	936.0	15
71	30_AIR	1	7:15:2	1008.0	15
72	30_AIR	2	7:15:2	773.0	15
73	45_CO2	1	7:15:2	1018.0	15
74	45_CO2	2	7:15:2	808.0	15
75	20_CO2	1	7:15:2	875.0	22
76	20_CO2	2	7:15:2	1024.0	22
77	30_CO2	1	7:15:2	859.0	22
78	30_CO2	2	7:15:2	923.0	22
79	30_AIR	1	7:15:2	826.0	22
80	30_AIR	2	7:15:2	890.0	22
81	45_CO2	1	7:15:2	1295.0	22
82	45_CO2	2	7:15:2	1133.0	22
83	20_CO2	1	7:15:2	1118.0	43
84	20_CO2	2	7:15:2	1018.0	43
85	30_CO2	1	7:15:2	1070.0	43
86	30_CO2	2	7:15:2	1210.0	43
87	30_AIR	1	7:15:2	1003.0	43
88	30_AIR	2	7:15:2	963.0	43
89	45_CO2	1	7:15:2	1680.0	43
90	45_CO2	2	7:15:2	1683.0	43
91	30_CO2	1	7:15:2	1202.0	65
92	30_CO2	2	7:15:2	1445.0	65
93	30_AIR	1	7:15:2	1198.0	65
94	30_AIR	2	7:15:2	1198.0	65
95	45_CO2	1	7:15:2	1313.0	65
96	20_CO2	1	7:15:2	1303.0	85
97	20_CO2	2	7:15:2	1037.0	85
98	30_CO2	1	7:15:2	1328.0	85
99	30_CO2	2	7:15:2	1363.0	85
100	30_AIR	1	7:15:2	1237.0	85
101	30_AIR	2	7:15:2	1318.0	85
102	20_CO2	1	7:15:2	1483.0	113
103	20_CO2	2	7:15:2	1438.0	113
104	30_CO2	1	7:15:2	1166.0	113
105	30_CO2	2	7:15:2	1273.0	113
106	30_AIR	1	7:15:2	1256.0	113

----------------------VARIABLE=FUDOM TYPES=CRUST----------------------

OBS	AIR	REP	FORMULA	VALUE	DAY
107	30_AIR	2	7:15:2	1137	113
108	20_CO2	1	7:15:2	1252	141
109	20_CO2	2	7:15:2	1247	141
110	30_CO2	1	7:15:2	1530	141
111	30_CO2	2	7:15:2	1255	141
112	30_AIR	1	7:15:2	1300	141
113	20_CO2	1	7:15:2	1008	169
114	20_CO2	2	7:15:2	1243	169
115	30_CO2	1	7:15:2	1346	169
116	30_CO2	2	7:15:2	1435	169
117	30_AIR	1	7:15:2	995	169
118	30_AIR	2	7:15:2	1154	169

Appendix G

FORMULA 7:1:2 FILE:CF7WRH MEANS WITHOUT R

TREATMENT

SAMPLE	DAYS	TIME	APPEARANCE CRST BROWN	SRMB COLOR	OVER INTEN	AROMA YEAST	ACRID	SOUR	CARME	RANCD	DRY	TEXTURE FIRM	SPRNG	CRMB	MOUTHFEEL DRY	GUM
	2	DAY 1	22	15	20	13	4	3		2	13	19	22	6	15	30
	2	DAY 1	25	28	25	15	6	6		2	16	20	25	10	16	28
-20CO2	8	WEEK 1	21	20	22	13	5	6		2	13	20	22	9	13	28
-20CO2	15	WEEK 2	20	16	23	13	4	3		2	12	18	18	9	15	26
-20CO2	36	WEEK 5	27	16	24	13	3	3		2	12	17	25	9	13	32
-20CO2	78	WEEK 11	26	16	26	13	3	3	3	3	17	20	28	8	15	31
-20CO2	120	WEEK 17	25	18	25	12	4	3	5	2	12	19	27	12	12	34
-20CO2		WEEK 22														
30AIR	8	WEEK 1	27	21	25	11	5	7		4	22	29	31	13	27	26
30 AIR	15	WEEK 2	22	20	29	11	9	7		7	28	36	36	22	29	18
30AIR	36	WEEK 5	17	23	30	11	7	8		8	27	35	37	25	34	18
30 AIR	78	WEEK 11	33	24	29	10	11	7	12	13	34	40	42	26	35	27
30 AIR	120	WEEK 17	26	29	34	8	16	17	11	14	29	35	33	25	31	25
30 AIR		WEEK 22														
30 CO2	8	WEEK 1	24	19	28	13	6	7		5	20	28	33	14	19	29
30 CO2	15	WEEK 2	25	20	26	10	4	7		6	25	31	36	24	25	23
30 CO2	36	WEEK 5	28	24	29	9	9	8		6	36	45	41	28	39	21
30 CO2	78	WEEK 11	37	27	30	10	11	9	10	10	40	43	43	28	42	26
30 CO2	120	WEEK 17	35	32	34	8	13	7	15	10	42	45	40	26	42	19
30 CO2		WEEK 22														
45 CO2	8	WEEK 1	33	24	29	12	10	8		6	27	29	36	14	27	27
45 CO2	15	WEEK 2	31	27	31	10	9	10		11	34	40	37	21	37	20
45 CO2	36	WEEK 5	37	41	35	8	10	8		16	41	47	46	27	42	13
20 CO2	78	WEEK 11	24	21	30	9	10	7	6	10	38	46	44	24	39	22
20 CO2	120	WEEK 17	27	22	29	9	9	11	11	11	40	40	39	32	41	23

Appendix H

FORMULA 7:1:2
TREATMENT

SAMPLE	DAYS	TIME	GRAIN	RATE HYDRT	SWEET	SOUR	BITTR	YEAST	CARML	CARDB RANCD	BURNT	BITTR	SWEET	PREF
			CRUMB FLAVOR								CRUST			
	2	DAY 1	5	30	17	5	3	10	4	5	10	9	10	33
	2	DAY 1	4	31	17	4	2	10	7	3	11	9	8	35
-20CO2	8	WEEK 1	6	32	13	4	4	11	7	2	10	6	7	34
-20CO2	15	WEEK 2	3	34	15	4	3	11	3	3	8	5	8	36
-20CO2	36	WEEK 5	7	38	18	3	2	10	4	4	12	6	7	35
-20CO2	78	WEEK 11	5	37	18	5	1	10	5	5	12	6	12	35
-20CO2	120	WEEK 17	7	39	18	3	3	12	9	4	14	7	8	35
-20CO2		WEEK 22												
30AIR	8	WEEK 1	6	24	15	5	3	8	5	4	9	10	7	31
30 AIR	15	WEEK 2	10	24	15	4	6	8	7	10	10	6	7	25
30AIR	36	WEEK 5	12	23	20	6	4	5	6	10	5	6	6	28
30 AIR	78	WEEK 11	20	22	20	5	4	7	17	13	9	8	11	16
30 AIR	120	WEEK 17	18	28	21	9	7	6	18	13	9	8	11	18
30 AIR		WEEK 22												
30 CO2	8	WEEK 1	9	28	18	4	3	9	5	4	7	9	10	30
30 CO2	15	WEEK 2	8	27	15	4	3	6	5	7	11	6	10	31
30 CO2	36	WEEK 5	19	21	16	7	6	8	6	12	7	8	7	21
30 CO2	78	WEEK 11	19	20	21	9	5	5	14	15	13	7	10	15
30 CO2	120	WEEK 17	18	23	18	9	6	8	21	17	12	8	11	14
30 CO2		WEEK 22												
45 CO2	8	WEEK 1	7	25	17	4	3	10	8	9	10	8	9	28
45 CO2	15	WEEK 2	13	21	16	6	4	7	7	11	11	7	10	21
45 CO2	36	WEEK 5	22	22	16	10	6	7	11	18	13	7	11	12
20 CO2	78	WEEK 11	17	22	21	7	4	8	10	9	9	8	12	17
20 CO2	120	WEEK 17	17	21	16	.10	5	7	13	14	10	6	11	19

FORMULA 6:1.5:2 FILE: CF6WRH WITHOUT
TREATMENT

SAMPLE	DAYS	TIME	APPEARANCE CRST BROWN	SRMB COLOR	OVER INTEN	YEAST	AROMA ACRID	SOUR	CARMEL	ANCD	DRY	TEXTURE FIRM	SPRNG	CRMB
	1	DAY 1	16	16	28	17	10	5		1	11	16	21	5
	1	DAY 1	21	18	23	12	5	7		2	9	16	17	7
-20CO2	8	WEEK 1	23	15	24	14	7	5		2	10	14	17	10
-20CO2	15	WEEK 2	29	15	22	12	3	3		3	9	14	22	10
-20CO2	36	WEEK5	23	13	26	15	5	3		3	10	15	19	13
-20CO2	78	WEEK11	23	12	25	17	5	3	3	3	12	17	19	9
-20CO2	120	WEEK17	22	13	25	17	3	4	5	2	12	16	25	11
-20CO2	168	WEEK22	27	13	26	15	4	3	8	2	11	13	18	15
30 AIR	8	WEEK 1	19	17	27	8	9	8		3	16	20	26	11
30 AIR	15	WEEK2	31	19	27	10	11	8		7	18	27	36	17
30 AIR	36	WEEK 5	25	20	31	9	7	6		7	25	30	33	25
30 AIR	78	WEEK 11	33	20	32	10	12	5	12	13	29	31	37	28
30 AIR	120	WEEK 17	32	26	34	6	14	10	12	12	34	37	40	29
30 AIR	168	WEEK 22	31	28	38	8	12	9	18	11	36	39	40	35
30 CO2	8	WEEK 1	27	19	28	13	7	6		2	17	27	28	14
30 CO2	15	WEEK 2	21	20	22	12	5	7		4	22	33	33	20
30 CO2	36	WEEK 5	29	19	27	8	7	6		8	26	33	35	22
30 CO2	78	WEEK11	39	26	32	7	7	7	18	13	34	37	41	29
30 CO2	120	WEEK 17	29	24	34	8	14	7	15	13	33	36	37	31
30 CO2	168	WEEK 22	35	31	35	9	13	9	13	10	34	36	39	33
45 CO2	8	WEEK 1	33	19	30	9	8	11		6	20	33	32	20
45 CO2	15	WEEK 2	31	24	29	11	8	7		7	23	30	40	24
45 CO2	36	WEEK 5	30	37	34	7	4	5		12	31	37	37	27
20 CO2	78	WEEK 11	29	19	26	8	8	4	8	7	33	40	40	30
20 CO2	120	WEEK 17	24	20	28	10	10	6	10	10	37	45	35	32
20 CO2	168	WEEK 22	35	20	27	9	7	10	8	10	29	32	31	28

Appendix I
FORMULA 6:1.5:2
TREATMENT

SAMPLE	DAYS	TIME	MOUTHFEEL DRY	MOUTHFEEL GUM	GRAIN	RATE HYDRT	CRUMB FLAVOR SWEET	CRUMB FLAVOR SOUR	BITTR	YEAST	CARML	CARDB RANCD	CRUST FLAVOR BURNT	CRUST FLAVOR BITTR	CRUST FLAVOR SWEET	PREF
	1	DAY 1	11	32	5	33	16	2	4	10	6	3	12	9	8	3[illegible]
	1	DAY 1	9	26	5	37	15	4	5	9	6	6	13	10	7	3[illegible]
-20CO2	8	WEEK 1	15	22	5	37	17	2	2	10	6	3	9	9	7	3[illegible]
-20CO2	15	WEEK 2	11	31	6	43	17	3	6	11	6	4	11	10	7	3[illegible]
-20CO2	36	WEEK5	10	31	7	39	15	4	2	13	5	3	9	9	6	3[illegible]
-20CO2	78	WEEK11	11	39	8	39	16	5	1	13	4	5	9	6	7	3[illegible]
-20CO2	120	WEEK17	12	37	6	42	18	7	3	13	5	3	10	4	8	3[illegible]
-20CO2	168	WEEK22	11	37	6	38	13	4								
30 AIR	8	WEEK 1	18	27	7	33	17	7	6	8	6	8	7	6	8	2[illegible]
30 AIR	15	WEEK2	22	27	9	31	15	3	7	7	7	8	13	12	6	2[illegible]
30 AIR	36	WEEK 5	26	20	14	29	17	6	5	6	7	10	8	5	10	2[illegible]
30 AIR	78	WEEK 11	36	27	15	25	18	5	7	7	13	16	10	8	8	2[illegible]
30 AIR	120	WEEK 17	40	23	21	22	17	13	6	5	14	15	10	9	9	1[illegible]
30 AIR	168	WEEK 22	37	23	20	20	16	10								
30 CO2	8	WEEK 1	16	22	6	31	16	2	4	12	6	6	8	8	7	3[illegible]
30 CO2	15	WEEK 2	24	23	9	29	14	4	5	10	6	7	7	8	8	3[illegible]
30 CO2	36	WEEK 5	33	24	14	22	16	4	3	6	8	11	10	8	7	2[illegible]
30 CO2	78	WEEK11	39	27	13	22	20	7	5	6	18	12	14	9	10	1[illegible]
30 CO2	120	WEEK 17	40	20	20	25	19	10	7	7	13	15	9	5	10	2[illegible]
30 CO2	168	WEEK 22	36	21	15	28	16	10								
45 CO2	8	WEEK 1	27	26	6	28	15	6	4	8	9	10	9	11	8	2[illegible]
45 CO2	15	WEEK 2	27	22	8	27	14	6	7	6	6	12	10	9	8	2[illegible]
45 CO2	36	WEEK 5	36	17	17	27	21	6	5	5	12	16	10	3	10	1[illegible]
20 CO2	78	WEEK 11	39	31	16	29	14	8	4	6	12	12	10	6	8	1[illegible]
20 CO2	120	WEEK 17	38	20	17	22	18	8	5	7	9	12	9	6	11	2[illegible]
20 CO2	168	WEEK 22	34	20	16	25	17	7								

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